STROBILUS INITIATION IN SLASH PINE IN RELATION TO SHOOT MORPHOLOGY AND PHYSIOLOGY OF THE TERMINAL BUD

By

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Strobilus initiation in slash pine (Pinus elliottii var. elliottii Engelm.) was examined in relation to characteristics of shoot morphology, carbohydrate and amino acid metabolism, and translocation of ^{14}C-labelled photosynthate in the terminal buds.

Grafted, 18-year-old slash pine clones in a seed orchard with different inherent potentials to produce female strobili were divided into two groups, abundant-flowering group (AFG) and poor-flowering group (PFG). Four types of terminal buds, with two types from each group, were examined: large buds (female-producing) and small buds (male-producing) in the upper and lower part of the crown, respectively, in AFG and large buds (vegetative) and small buds (male-producing) in the upper and lower part of the crown, respectively, in PFG.

Histochemical examination of terminal buds showed that initiation of male strobilus primordia in Gainesville,

Florida, started in early July, whereas that of female strobilus primordia started in early September.

Shoot morphology and shoot vigor appeared to determine the future sexuality of the shoot. A positive correlation was observed between the total weight of current-year needles per shoot and number of female strobili produced the following year, whereas number of male strobili was negatively correlated with the total weight of current-year needles. Thus, a transition from male to female flowering was associated with increasing vigor (number of needles) of the shoot.

Terminal buds of four different types were collected from late July to early September to analyze free (75 percent ethanol-soluble) sugars (by gas chromatography) and free amino acids (by an automatic analyzer). Sugar concentration in the large buds (female-producing) of AFG was greater than in the small buds (male-producing) of the same group. Amino acid concentration in the large buds of AFG was lowest among the four types of terminal buds, suggesting a temporary reduction in the metabolic activity of the female-producing buds. Initiation of female strobilus primordia was associated with a high sugar to amino acid ratio. Arginine concentration in the large buds of AFG was lower than in other three types of buds, suggesting a minimal or negative role of arginine in the initiation of female strobilus primordia.

When current-year needles were exposed to $^{14}CO_2$ in late August, translocation of ^{14}C-labelled photosynthate from

needles to the terminal buds was much slower in the large buds of AFG than in the other three types of buds, due to reduced capacity of these buds to act as metabolic sinks and a large demand of actively developing second-year cones for photosynthate. The amount of ^{14}C-labelled photosynthate found in the male-producing buds in late August and early September seemed to be related to the number of developing male strobilus primordia. When radioactivity in alcohol extract of the bud tissue was partitioned into various fractions by ion exchange chromatography, relative distribution of radioactivity in hexane-soluble, sugar, amino acid, and organic acid fractions showed no significant difference among the four types of buds.

CHAPTER I
INTRODUCTION

Research on flower induction has concentrated primarily upon herbaceous plants which respond consistently to specific treatments such as changes in photoperiod or vernalization. However, such treatments seem to be ineffective in the induction of flowering of conifers, even though photoperiod affects the duration of shoot elongation and time of formation of winter buds (Jackson and Sweet, 1972; Kozlowski, 1971). Physiological processes involved in the initiation and determination of floral structures in pines are poorly understood. In addition, the size and time required to attain maturity make trees difficult organisms for flowering research.

The complexity in the flowering of pines is reflected by diverse treatments employed to induce or promote flowering. Some treatments (such as fertilization, irrigation, and thinning) improve the general site conditions. Other treatments (such as girdling, induced drought, nitrogen starvation, root restriction, frost damage, etc.) induce a severe shock to the plants. It is interesting to note how these two contrasting groups of treatments, one favoring and the other adversely affecting plant growth, bring about

the same effect in stimulating flowering. There has been no adequate explanation for the effects of the above treatments on the internal physiological processes related to the transition from vegetative to reproductive growth.

Moisture stress during the early summer growing season positively influences floral bud initiation in conifers. Shoulders (1973) suggested that decreased vegetative growth during this period might allow accumulation of carbohydrates in the shoots, thus favoring differentiation of reproductive structures.

Physiological explanations for stimulation of flowering by nitrogen have shown that fertilizing with ammonium nitrate increased free arginine and total free amino acids in needles and twigs (Barnes and Bengtson, 1968). Ebell and McMullan (1970) proposed that this arginine-type metabolism was quantitatively associated with female cone production.

In contrast to the positive effect of nitrogen fertilization on flowering, there are some reports on stimulation of flowering by nitrogen deficiency (Lyr and Hoffmann, 1964; Kuo, 1973; Kamienska et al., 1973). The nitrogen deficiency was associated with a significant increase in less-polar gibberellins (Kuo, 1973). Pharis (1976) proposed that cultural treatments known to promote flowering in conifers might alter gibberellin metabolism by interfering with interconversion of gibberellins within the plant. However, the effect of cultural treatments on carbohydrate and nitrogen metabolism is not well known.

Shoot vigor has been related to the capacity of the shoot to produce female strobili. Only dominant trees in a pine stand produce female cones (Fowells and Schubert, 1956). In Pinus elliottii, Varnell (1970) showed that cone-bearing secondary branches had longer, thicker shoots and terminal buds than nonbearing branches, indicating that morphology of terminal buds was directly related to sexuality of the shoot. However, physiological implication of the size of the buds in flowering was not discussed.

Demands for seeds from known sources with high quality and genetic superiority are increasing in forest management programs. To meet these demands, pine seed orchards have been established using grafted trees which were genetically improved for disease resistance, growth, and form. Pines, however, normally do not produce seed during their juvenile stage of development. Seed production from the progeny of two selected parent trees may not be expected during this juvenile phase. Hence, at least a decade is required between generations in breeding programs. In addition to the problem of juvenility, inherent variations in fruitfulness and irregularity in flowering make it difficult for tree breeders to predict the size of cone crops.

The goal of research on flowering is to establish basic principles under which foresters may be able to regulate cone production at will. Thus, understanding of the processes involved in the early stages of transition to a reproductive phase and floral bud initiation is of primary importance.

To understand the physiological processes involved in floral bud initiation, two groups of clones of slash pine (Pinus elliottii var. elliottii Engelm.) with different inherent potential to flower were selected from a seed orchard. The objectives of the study were:

1) to establish relationships between shoot morphology and strobilus production;
2) to determine nutritional status and the distribution of certain metabolites in the terminal buds during the period of floral bud initiation;
3) to identify the physiological characteristics related to differences in flowering potential between abundant- and poor-flowering trees.

CHAPTER II
LITERATURE REVIEW

Biological Aspects of Flowering in Conifers

A flower is a determinate sporogenous shoot bearing carpels (Hillman, 1962). Such a definition defined for angiosperms is not applied to the sporogenous strobili of gymnosperms. However, foresters commonly refer to the ovulate and staminate strobili as female and male flowers, respectively. Jackson and Sweet (1972) proposed to redefine a flower simply as "a determinate sporogenous shoot" so that sporogenous strobili of gymnosperms would correctly be called coniferous flowers. In this review the term "flowering" will be used to refer to the developmental process resulting in the production of sporogenous structures in both angiosperms and gymnosperms.

In Pinus, staminate and ovulate strobili are borne in separate structures on the same tree. Staminate strobili are generally formed in the lower portion of the crown, while ovulate strobili are formed in the strong leading branches of the upper crown. This suggests that shoot vigor is involved in sex determination (Wareing, 1958).

Staminate strobili appear to be initiated earlier during the growing season than ovulate strobili. In Pinus elliottii,

staminate strobili were initiated at the end of June in northern Florida as distinct swellings in axils of partially formed cataphylls (Mergen and Koerting, 1957). By the middle of September several layers of hood scales were formed by the meristematic area of the primordium and rudimentary microsporophylls were distinguishable. By mid-December microsporangia were filled with microspore mother cells, and in late January growth resumed and mature pollen grains were released during February.

Ovulate strobili of Pinus elliottii were believed to be initiated in late August in Florida (Mergen and Koerting, 1957). They first became noticeable as slight protuberances in the axil of a developing cataphyll near the tips of vegetative buds. In late November bract scale primordia were initiated and ovuliferous scales were formed in late December. Unlike the staminate strobili, the ovulate strobili continued growth during the winter and became ready for pollination in early February.

In pines, once floral bud primordia are initiated in a growing season, it requires an additional two growing seasons to produce fully mature seeds. Thus, the seed crop of a given year reflects not only the degree of abortion of female strobili in the same year and the success of pollination in the previous growing season, but also the amount of floral bud primordia initiated two years earlier. For example, Shearer and Schmidt (1971) observed that in Pinus ponderosa 27 percent of the potential cone crop survived

the first year of development and only 6 percent reached maturity and shed their seeds. Thus, good cone crops occur once every few years in certain species. This periodicity in flowering has also been known to horticulturists as biennial or alternate bearing and seems to be a common phenomenon in woody plants.

The involvement of the three growing seasons for the full development of floral structures and the possible effects of climatic factors during any period of these growing seasons increases the difficulty of understanding the mechanism of flowering in pines. Furthermore, there seems to be no unique treatment or stimulus which induces woody plants, and particularly conifers, to flower consistently. Romberger (1967), based on the diversity and complexity of flowering phenomena of woody plants, suggested that the concept of "floral induction" as a single event seemed to be inadequate to describe the multistep nature of flower initiation in woody plants. He proposed the term "floral determination" to describe such situations in woody plants.

This review is limited to topics related to floral bud initiation in conifers. As the main body of the experiments of this dissertation is concerned with the nutritional status related to shoot morphology and consequent flower production, factors which affect the nutritional balance of trees will be critically analyzed.

Photoperiod

In most fruit and forest trees the effect of photoperiod on flowering is not clearly understood (Wareing, 1959; Matthews, 1963; Jackson and Sweet, 1972). Mirov (1956), after studying the flowering behavior of over 38 species of Pinus from a range of different provenances, concluded that pine species behave not as long-day or short-day plants, but as neutral plants whose flowering is not affected by length of day. Many pine species, however, exhibit growth responses to photoperiodic treatments.

A negative effect of photoperiodic treatment under interrupted nights on Pinus attenuata was observed by Lanner (1963), and reduction of the growth period of Picea mariana under short days was reported by Fraser (1966). Working with Pinus densiflora, Goo (1968) observed that seedlings under 8-, 16-, and 24-hour photoperiod did not have any flowers while those under natural day-length produced female flowers. Marked elongation of winter buds of seedlings under 16- and 24-hour photoperiod suggested that potential strobilus initials were transformed into dwarf-shoot initials.

There are a few reports on the positive effects of photoperiod in conifers. Longman (1961) observed that young potted Pinus contorta produced more female flowers under short days than controls under natural day-length. In Pinus thunbergii and P. densiflora short-day treatment before chilling and continuous light after chilling were the conditions most favorable to cone development (Katsuta, 1975).

The relationship between date of flushing and flowering in Pinus banksiana was reported by Larson (1961) who induced potted seedlings to flush at different times in the spring by removing them from a cold room in early or late April. He noticed increased ovulate strobilus formation in the seedlings removed earlier in the spring with shorter day-length.

In Pinus elliottii a time lag of about 8 weeks was noticed between the first initiation of male strobilus primordia and that of female primordia (Mergen and Koerting, 1957). Male strobilus primordia were initiated in late June during long days, whereas female primordia started to initiate in late August when day-length was decreasing. Thus, it is possible, as suggested by Giertych (1967), that male strobilus primordia might be initiated under the influence of long days, whereas female primordia might be initiated under the influence of short days.

Pharis and Morf (1967) reported that induction of flowering by gibberellins in Thuja plicata had a quantitative requirement for long days (16 hours of light). When the induced seedlings were kept under long days, the number of reproductive apices was about 6 times those under short days. Further study (Pharis et al., 1969) showed that subsequent development of the strobili after the initial induction by long days required a sequence of short days and long days.

Shoot Morphology and Tree Vigor

The amount of light received by individual trees seems to influence their vigor and their capacity to produce female cones. Only dominant trees in a pine stand regularly produce female cones (Fowells and Schubert, 1956), indicating the importance of tree vigor in cone production. In a Pinus ponderosa stand, large vigorous, isolated trees were the best cone producers in terms of seed quantity and quality, and frequency of bearing (Larson and Schubert, 1970). Andersson and Hattemer (1975) reported that the number of female strobili was positively related to tree size for Pinus sylvestris. Reproductive maturity in Pinus taeda was related to cumulative total height growth (Schmidtling, 1969). Stem diameter, however, does not seem to be closely related to cone production (Grano, 1957; Varnell, 1976a), but a strong correlation existed between cone production and size of crown (Grano, 1957; Cappelli, 1958).

Within a single tree, branch vigor appears to determine the future locations of female flowers (Wareing, 1958; Varnell, 1970, 1976b). In a systematic study on the distribution of female flowers around the crown of Pinus sylvestris, Wareing (1958) observed that the reduction (measured by extension growth) in the vigor of the shoot was associated with a reduction in the formation of female cones and the appearance of male cones. Varnell (1970), working on the relationship between vegetative branch growth and subsequent production of female cones in Pinus elliottii, found that

average length and diameter of secondary branches which bore female cones were larger than those of branches which did not produce female cones. Thus, it was possible to predict the future sites of female strobilus initiation. Branch volume in Pinus taeda (Thorbjornsen, 1960) and in P. resinosa (Rim and Shidei, 1974) were significantly correlated with female cone production. Branch order and size of branch also seemed to be related to female flowering. Anikeeva and Minina (1959) reported that in Picea abies first- and second-order branches in the upper crown bore female flowers, whereas third- and fourth-order branches in the lower and middle crown had a strong tendency to bear male flowers.

Physiological implications of shoot morphology and vigor of the trees in flowering are not understood, but are believed to be related to the carbohydrate nutrition of the shoots.

Inherent Fruitfulness

Individual variability in growth rate, flowering, and other characteristics is very common to pines. It is often difficult to distinguish between inherent and environmental influences on pine flowering. As emphasized by Squillace (1966) pines have considerable genetic diversity and this different genetic potential of individual trees is responsible for the different responses of neighboring trees of the same species to a particular set of environmental conditions (Pritchett and Goddard, 1967).

Barnes and Bengtson (1968), while making observations on the growth and flowering of clones of Pinus elliottii under intensive culture, noted the pronounced inherent differences among clones in flowering. In seed production areas where trees are free of crown competition, cone yields vary widely among trees (Webb and Hunt, 1965) and this seems to be the major problem in seed collection, because phenotypes selected for good growth and form vary so widely in seed production that sometimes seed collection is not feasible from certain low producers (Van Haverbeke and Barber, 1964). Greene and Taylor (1974) also noted that flower production by early flowering clones was significantly influenced by the fertilizer treatments, while flower production by non-flowering clones was not affected.

Dorman (1976) emphasized that evidence of good inherent seed production should be required in selection for clonal seed orchards. In a Pinus elliottii seed orchard, Beers (1974) observed that, among 36 representative clones, cone production of the most productive clone exceeded the least one by a factor of about 15, and one-fifth of the clones produced 55.6 percent of the annual seed production. Since the most important factor influencing fruitfulness in conifers seems to be the inherent ability of the individual tree to flower (Schmidtling, 1974; Shoulders, 1967), it is necessary to consider the inherent fruitfulness of study trees in experimental designs to reduce the random variations in flowering response. Broad-sense heritability of fruitfulness has

been estimated to be 0.5 in Pinus elliottii (Varnell et al., 1967) and 0.4 to 0.7 in P. taeda (Schmidtling, 1974).

The physiological explanations for the difference in inherent fruitfulness in conifers seem to be lacking. Smith and Stanley (1967) reported that high cone producing trees had higher nitrogen content in needles than low cone producing trees in response to nitrogen fertilizer. They suggested the involvement of a metabolically stable nitrogen compound in the cone production response to nitrogen fertilizer.

Silvicultural Induction of Flowering in Conifers

Thinning

The most successful treatment to consistently stimulate flowering in pines may be thinning or releasing from competition. Thinning increased, markedly in most cases, the cone production in Pinus echinata (Phares and Rogers, 1962; Yocom, 1971), P. elliottii (Halls and Hawley, 1954), P. monticola (Barnes, 1969), P. palustris (Allen, 1953), P. resinosa (Godman, 1962; Cooley, 1970), P. radiata (Eldridge, 1966), P. taeda (Wenger, 1954; Bilan, 1960; Allen and Trousdell, 1961), and Pseudotsuga menziesii (Reukema, 1961).

There seems to be a quantitative relationship between the degree of thinning and the degree of stimulation. Godman (1962) found in Pinus resinosa that the percent of trees bearing cones in 6 different levels of thinning was directly proportional to the degree of thinning, with 96 percent of

the trees bearing cones in the heaviest thinning, and only 19 percent in the lightest thinning.

The stimulating effect of thinning is believed to be associated with availability of light, water, and nutrients. However, physiology of the tree in relation to food and nutrient supplies after thinning has not been studied. It is possible that the balance between carbohydrate and nutrient supplies may be disturbed after the thinning due to a sudden change in environment, and that this sudden change may cause a shift to the reproductive phase.

Girdling

Stem or branch girdling and strangulation (with wire around the stem) have been used to promote cone production in conifers. For example, girdling increased female cone production by four times in Pinus echinata (Bower and Smith, 1961) and by seven times in Pseudotsuga menziesii (Ebell, 1971). When a heavy thinning was followed by girdling, flowering increased by the factor of 57 in 49-year-old Larix leptolepis seed trees (Asakawa et al., 1966).

The effectiveness of strangulation in stimulating flowering seems to be less than that of girdling. Melchior (1961) observed that strangulation was much less effective than girdling in flower induction in Larix leptolepis. In Pinus palustris strangulation did not stimulate cone production, while girdling increased cone production by about two times (Mann and Russell, 1957). Banding with a metal strip around

a branch or trunk seems to have a much lower effectiveness than girdling or strangulation. The decreasing effectiveness in the order of girdling, strangulation, and banding suggests that the severity of restriction or interference with downward movement of food in the phloem determines the degree of stimulation (Bilan, 1960).

A physiological explanation for the stimulating effect of girdling is based on the increased carbohydrate contents above the girdle. Ebell (1971) found that both sugars and starch accumulated in shoots after girdling, even though food reserves were weakly related to reproductive bud survival in Pseudotsuga menziesii. In Cryptomeria japonica the contents of reducing sugars, total soluble carbohydrates and starch in the shoots increased after girdling, while the contents of water and nitrogen decreased, resulting in a sharp increase in the carbohydrate to nitrogen ratio (Hashizume, 1970). A high correlation was recognized between the number of flower buds formed and the amounts of these chemical constituents.

Root Restriction

Root growth can be limited by such treatments as root pruning, root restriction, and transplanting. These treatments have been shown to stimulate cone production in some conifers. Root pruning in Pinus elliottii (Hoekstra and Mergen, 1957), P. strobus (Stephens, 1961, 1964) and P. taeda (Gregory and Davey, 1977) increased female flowering.

No effect of root pruning on flowering was reported in Larix leptolepis (Heitmüller and Melchior, 1960) and Pseudotsuga menziesii (Melchior, 1968).

An interesting observation on the flowering of transplanted trees was reported by Quirk (1973). He lifted 100 trees of 10-year-old Pinus resinosa in April, pruned the roots, and planted them into tubs. The confined trees produced an average of 29 and 108 ovulate strobili per tree the second and third growing season, respectively, while unlifted trees in the plantation produced essentially no cones. The lifted trees also showed an extreme profusion of staminate strobili in the lower half of the crown. Another instance of profuse flowering after transplanting was observed in grafted Pseudotsuga menziesii. Silen (1973) moved seed orchard trees in early July and found that lifted trees produced an average of 23 cones compared with near zero for corresponding unmoved ramets. The fact that considerable root pruning and subsequent drought symptoms resulted from the operation suggested that cause of the stimulated flowering could be associated with water stress.

Moisture Stress

In Pinus, Picea, and Larix whose floral buds are believed to be initiated between July and August (Mergen and Koerting, 1957; Fraser, 1966; Hashizume, 1973), prolific female flowering seems to be associated with abundant rainfall in the spring (March to May) and low rainfall in early summer (June

to August) of the previous year (Fraser, 1958; Yanagihara et al., 1960; Shoulders, 1967, 1973; Rehfeldt et al., 1971; Grano, 1973). The requirement of dry summers for prolific female flowering indicates that floral differentiation is favored by moderate moisture stress which reduces vegetative growth. A correlation of hot and dry summer with mast production the following year in Fagus sylvatica has been well documented (Matthews, 1955; Holmsgaard and Olsen, 1961). Shoulders (1973) suggested that continuous photosynthesis in summer while vegetative growth is curtailed by moisture stress favors accumulation of readily available carbohydrates at the time of flower bud initiation. A relatively high level of carbohydrates has been proposed to be necessary for the initiation of flower buds (Kramer and Kozlowski, 1960).

Induced moisture stress was shown to be beneficial in cone initiation. Ebell (1965, 1967) induced drought in potted Pseudotsuga menziesii during the period of floral bud initiation and found increases in both male and female flowers. He suggested that floral initiation in long-lived species with pronounced periodicity may require critically timed moisture stress that is temporarily unfavorable for vegetative growth.

Irrigation experiments (Dewers and Moehring, 1970) showed that continuous irrigation from April through September in a Pinus taeda seed orchard did not increase female flowering, whereas trees subjected to April through June irrigation followed by July through September drought produced

a significantly greater conelet crop. The soil moisture regime manipulated by irrigation in their experiment seems to be similar to a climatic requirement of abundant spring rainfall followed by low summer rainfall. Barnes and Bengtson (1968) and Bengtson (1969) observed that irrigation of Pinus elliottii in a seed orchard during the growing season (from March to November) reduced female flowering, while increasing male flowering. Irrigation of Pinus monticola during dry summers also increased female flowering (Barnes and Bingham, 1963).

Physiological Processes Related to Flower Induction in Conifers

Nutrition: Carbohydrates and Nitrogen

Kramer and Kozlowski (1960) proposed that a high level of carbohydrates was required for flower initiation in woody plants. Factors related to the vigor of the trees, such as full sunlight, dominance, thinning, and branch pruning are believed to directly improve the carbohydrate status of trees through enhancing photosynthesis. Despite these implications, there is no unequivocal evidence that carbohydrates play a direct role in flower induction. Eis et al. (1965), studying annual ring width as a measure of carbohydrate level, found no relationship between flower production and the level of carbohydrates. Even girdling which has been proposed to increase carbohydrate levels above the girdle did not significantly increase the carbohydrate concentration in Pseudotsuga menziesii (Ebell, 1971).

Factors related to the uptake of mineral nutrients, such as soil fertility, fertilization, and irrigation improve the mineral nutrient status of trees. When nitrogen fertilizer was applied to Pinus elliottii, it increased nitrogen content of the shoots, whereas carbohydrate levels were not affected (Barnes and Bengtson, 1968). Fertilizing with ammonium nitrate doubled female flower production and increased free arginine, total free amino acids, and total nitrogen in needles and twigs (Barnes and Bengtson, 1968). A large clonal difference in flowering response was noticed, but it was not correlated to the level of nitrogen content, arginine, or total amino acids in twigs. Ebell and McMullan (1970) noticed large accumulations of basic amino acids, notably arginine, and guanidino substances after Pseudotsuga menziesii was fertilized with nitrogen. They proposed that this arginine-type metabolism is quantitatively associated with cone production.

The response of male and female flowering to nitrogen fertilization seems to be different. In most cases female flowering was stimulated by nitrogen applications; whereas male flowering had either a nil or negative response (Barnes and Bengtson, 1968; Giertych and Forward, 1966). This difference in response seems to indicate different requirements of nitrogen nutrition for initiation of the two strobilus types.

The time of fertilizer application appears to be critical to give best response. Stoate et al. (1961) observed

the increase in cone production in Pseudotsuga menziesii only when fertilization took place at the time of bud break. Fertilization with nitrogen two weeks before or after bud break did not increase cone production. Ebell (1972) also observed the best response in the same species when nitrogen was applied at the start of bud break. Schmidtling (1974, 1975) showed that female flowering of Pinus taeda in response to nitrogen fertilizer was most abundant when fertilizer was applied in August when female primordia are believed to be initiated. Schmidtling (1974) interpreted the increase in flowering to be the result of enhanced photosynthesis followed by an accumulation of carbohydrates and nitrogen favorable for flower induction. He also stated that application of nitrogen in August was too late to stimulate vegetative growth but still enhanced photosynthesis which was essential for flower induction.

An interesting observation concerns the significance of the form of nitrogen in applied fertilizer. Cone production in Pseudotsuga menziesii increased up to 7 times (Ebell and McMullan, 1970) or 10 times (Ebell, 1972) more than untreated controls when 1,600 pounds per acre of nitrate nitrogen were applied during early bud break; whereas, ammonium nitrogen applied at the same rates and times was ineffective for stimulation of cone production. With applications of these two types of fertilizer there were no differences in the rate of accumulation of total nitrogen in buds and foliage. Ebell (1972) concluded that different responses

were not due to relative availability or rate of uptake of these two forms of nitrogen or improved nutrition, but due to "a specific chemical stimulation from critically timed changes in type of nitrogen metabolism."

In contrast to the positive effect of nitrogen on flowering, there have been some reports of positive effects of nitrogen deficiency on flowering. Kuo (1973) and Kamienska et al. (1973) observed precocious flowering in Cupressus arizonica seedlings induced by nitrogen deficiency. Lyr and Hoffmann (1964) also were able to induce 3-year-old Cryptomeria japonica seedlings to flower by artificially induced nitrogen deficiency. Giertych (1975), after examining the mineral distribution in the crown, reported that female flowers in Pinus sylvestris were initiated under the conditions of limiting mineral supplies including nitrogen.

A high carbohydrate to nitrogen (C/N) ratio was proposed to promote flowering in fruit trees, while a low C/N ratio favors vegetative growth (first proposed by Kraus and Kraybill in 1918 and cited in Kraus, 1925). Even though much of the past work has supported this theory, there is still uncertainty whether a high C/N ratio is a cause of flowering or a consequence of reduced vegetative growth during the period of flower initiation. High C/N ratios were associated with flowering of nitrogen-starved Cupressus arizonica (Kuo, 1973; Kamienska et al., 1973) and Cryptomeria japonica (Lyr and Hoffmann, 1964). Girdling (Hashizume, 1970) and gibberellin treatment (Hashizume, 1961) which

stimulated flowering in the latter species increased carbohydrate contents and decreased nitrogen level, elevating the C/N ratio. A high correlation was also recognized between the number of flowers formed and the carbohydrate and nitrogen contents.

One of the experimental observations contrary to the C/N theory appears to be the stimulating effect of nitrogen fertilization on female flowering. Nitrogen fertilization promotes female flowering in Pinus, Pseudotsuga, Picea and some angiosperm species (Matthews, 1963). Nitrogen fertilization increased foliar nitrogen contents while this treatment had no effect on carbohydrate levels, thus decreasing C/N ratio (Barnes and Bengtson, 1968). It is uncertain, however, whether nitrogen is a limiting factor in flower initiation, judging by the experimental observations of flower induction by either nitrogen fertilization or induced nitrogen deficiency.

Growth Regulators

Auxins. The relative distribution of male and female strobili within the crown seems to suggest the involvement of growth regulators, particularly auxins, in sexuality. When Pinus sylvestris trees reach maturity, the first reproductive structures, female strobili, are formed on the strong leading shoots near the tree apex. The association of femaleness with shoot vigor is believed to be controlled

by auxins produced from the apex (Wareing, 1958). A gradient of sexuality along the branch observed in Thuja plicata seems to reflect the auxin gradient originating in the shoot apex, with higher auxin levels near the apex favoring femaleness (Pharis, 1976).

Hashizume (1969) extracted auxins from Pinus thunbergii and P. densiflora, and found that auxin activities in the female buds collected from the upper part of the crown were higher than in the male buds collected from the lower portion of the crown. Pharis (1976) proposed that a balance between auxins and gibberellins is the most critical factor in sex determination, and that high auxin to gibberellin ratios promote femaleness while lower ratios promote maleness. This point is also supported by Kopcewicz et al. (1977) who found a high level of auxins and a low level of gibberellins in the female-producing buds during the period of floral bud initiation. Duff and Nolan (1958), on the other hand, stated that flower initiation is associated with low auxin levels, based on the fact that initiation of floral bud primordia occurred following the period when shoot extension growth ceased. Giertych and Forward (1966) also noticed that growth promoters were in low concentrations during the period of floral bud initiation.

Exogenous applications of auxins increased the proportion of female flowers in Larix leptolepis (Hashizume, 1967, 1973), but this caused some injuries to the shoots (Hashizume, 1967). Negative effects of auxin application on

flowering of conifers are commonly reported (Mann and Russell, 1957; Hashizume, 1959; McLemore, 1975; Bleymüller, 1976; Bonnet-Masimbert, 1971; Brune, 1973).

Despite the implications mentioned above, the role of auxins in flower initiation in woody plants is uncertain.

Gibberellins. The role of gibberellins (GAs) in flowering of conifers seems to be positive compared to that of auxins. Since Kato et al. (1958) first demonstrated that flowering in Cryptomeria japonica could be induced by gibberellin application, gibberellins, in most cases GA_3, have been used successfully to stimulate flowering in members of the Cupressaceae and Taxodiaceae (Pharis and Ross, 1976). Unfortunately, the success in members of the above two families was not readily repeated in the Pinaceae which include commercially more important species. However, recently Pharis and his colleagues (Pharis et al., 1976) have shown that some Pinaceae respond to the less-polar GAs, such as GA_4, GA_5, GA_7, and GA_9, which are believed to be precursors to other biologically active, more oxidized GAs. The species in the Pinaceae which responded include Pinus taeda, P. contorta, and Pseudotsuga menziesii (Pharis, 1975).

The levels of various endogenous GAs were reported to change when certain environments are modified. This change in endogenous GAs was related to subsequent flowering. Kuo (1973) induced Cupressus arizonica seedlings to flower under nitrogen deficiency and analyzed levels of endogenous GAs. He found a significant increase in less-polar GAs, while

polar GAs decreased substantially. The same trend was also observed in the seedlings of Pseudotsuga menziesii induced to flower by water stress, nitrate fertilization, and girdling (Pharis, 1976; Ross and Pharis, 1976). Thus, increased levels of the less-polar GAs were correlated with a flowering situation. Conversely, a vegetative state was associated with high levels of GA_3 and other polar GAs (more oxidized GAs). Since the biosynthetic sequence of oxidation of GAs in higher plants is from less-oxidized to more-oxidized forms, Pharis (1975) proposed that cultural treatments known to promote flowering in conifers might be doing so by interfering with interconversion of GAs within the plant, thus allowing a build-up of the less-oxidized precursor GAs which in turn promote sexual differentiation.

His hypothesis is further supported by the effectiveness of GA_4 and GA_7 mixture and GA_9 in promoting flowering in Pinaceae. For example, Ross and Pharis (1976) observed that biweekly applications of 400 µg $GA_{4/7}$ per branch of Pseudotsuga menziesii between late March and late June gave a 5-fold increase in ovulate and 3-fold increase in staminate strobilus production over untreated controls. N-Benzyladenine and 2,3,5-triiodobenzoic acid applied in combination with GAs had no consistent effect on strobilus production. The time of GA application appears to be critical and applications should be made in that period of time when sexual differentiation takes place (Pharis, 1976).

Photoperiod appears to interact with exogenous GAs in determining sexual differentiation. Pharis et al. (1970) showed that induction of male strobili in Cupressus arizonica by exogenous GA_3 appeared to have a quantitative requirement for long days. The induction was in part under photoperiodic control as shown by interruption of the dark period. Glenn (1973) observed that when seedlings of this species were grown under daylengths of 4, 8, 12, 16, and 24 hours, those seedlings under the longer photoperiods required less exogenous GA_3 to initiate flowering. An examination of the endogenous GAs of control plants which did not receive exogenous GA_3 revealed a massive increase in endogenous GA_3 and other more polar GAs as daylength increased. His experiment suggested that C. arizonica, in a juvenile phase, may respond to photoperiod but is not able to produce enough GAs to induce flowering. In fact, Pharis et al. (1965) found that young seedlings of this species required more exogenous GA_3 to flower than older ones. Thus, Pharis and Morf (1968) suggested that the juvenile phase in this species may be that period during which endogenous GAs have not yet attained sufficient concentration to cause flowering, with GAs being preferentially utilized for vegetative growth.

The effects of GA application on the carbohydrate and nitrogen metabolism in relation to induced flowering is uncertain. Hashizume (1961) noticed that GA treatment which induced flowering in Cryptomeria japonica caused changes in carbohydrate and nitrogen contents in the shoots. Total

nitrogen decreased after the treatment, but reducing and non-reducing sugars, and soluble and insoluble carbohydrates increased after the treatment. Consequently, C/N ratio in the treated shoots was higher than that of untreated control.

One of the interesting phenomena in GA metabolism is the increased endogenous levels of less-polar GAs caused by induced nitrogen deficiency (Kuo, 1973) and by nitrate fertilization (Pharis, 1976). Induced nitrogen deficiency will decrease nitrogen content in the tissue, thus elevating C/N ratio, while nitrate fertilization will increase the tissue contents of nitrogen, thus lowering C/N ratio. These two treatments, however, brought about a similar increase in less-polar GAs. It is likely that GAs may play a role in measuring the balance between carbohydrates and nitrogen by means of interconversion of GAs. Increased levels of less-polar GAs in water-stressed and girdled trees (Pharis, 1976) also support this idea. Thus, gibberellins appear to play a central role in flower induction in conifers and their effect on carbohydrate and nitrogen metabolism in relation to flowering need further investigation.

CHAPTER III
SHOOT MORPHOLOGY AND OVULATE STROBILUS PRODUCTION

Introduction

The induction of male and female strobili in pines has been related to certain anatomical and morphological characteristics as measures of shoot vigor (Wareing, 1958; Varnell, 1970, 1976b). In Pinus sylvestris, Wareing (1958) observed that the reduction in the vigor of the shoot (measured by extension growth) was associated with a reduction in the formation of female cones and appearance of male cones. Varnell (1970) found that in Pinus elliottii the secondary branches that produced female strobili had longer and thicker shoots and terminal buds than nonflowering branches, indicating that morphology of terminal buds is directly related to sexuality of the strobili produced on the shoot.

Physiological implications of shoot vigor in flowering of pines are not known. The significance of shoot vigor and terminal bud size in cone production suggests that the number of needles on the shoot, size of metabolite-transporting tissue (phloem), and the magnitude of the metabolic sink in the terminal buds might form an integrated system which regulates the carbohydrate availability to the terminal buds.

Objectives of this study were to identify the relationship between shoot morphological and anatomical characteristics and initiation of female flower primordia in Pinus elliottii.

Materials and Methods

Description of the Seed Orchard and Flowering Behavior

The School of Forestry at the University of Florida established a slash pine (Pinus elliotti var. elliottii Engelm.) seed orchard in 1956 using vegetatively propagated (grafted) clones. The seed orchard is located at the Horticultural Unit, University of Florida, Gainesville, Florida. Some of the clones have produced cones since 1960, and the history of clonal flowering behavior in this orchard was reported by Goddard (1964). Clones with a history of abundant cone production consistently produce more cones than those with a history of poor flowering. Table 1 shows the average number of female cones produced from 1960 to 1965 in 10 different clones with 2 ramets each. These clones were chosen for study in this experiment. The flowering behavior of two ramets in each clone also showed consistency, indicating the strong genetic influence on flowering potential. The classification of these clones into two groups, "abundant-flowering" and "poor-flowering," was based on this history.

Table 1. Average annual number of female stobili produced per tree in 10 different clones of Pinus elliottii during the period of 1960 to 1965.

poor-flowering group			abundant-flowering group		
clone No.	ramet 1	ramet 2	clone No.	ramet 1	ramet 2
	cones/ tree	cones/ tree		cones/ tree	cones/ tree
59-56	13	9	243-55	90	80
123-56	4	5	289-55	92	64
118-56	6	14	116-56	71	90
248-55	13	9	113-56	80	74
139-56	18	9	133-56	68	74

Shoot Vigor and Strobilus Production

Five clones with a history of abundant female cone production (abundant-flowering group) and five clones of poor female production (poor-flowering group) were used in this experiment (see Table 1). In the abundant-flowering group two types of terminal buds within a tree were selected as described below:

a) large buds - terminal buds located in the upper part of the crown with an above average diameter (at least one standard deviation above the tree mean) and with one or more current-year female strobili on the shoots (the buds in which female strobili are expected to be produced the following year).

b) small buds - terminal buds located in the lower part of the crown with a below average diameter (at least one standard deviation below the tree mean) and with no female strobili on the current and previous year's shoots (the buds in which male strobili but not female strobili are expected the following year).

In the poor-flowering group two types of the terminal buds (comparable to those of abundant-flowering group) within a tree were also compared:

a) large buds - terminal buds located in the upper part of the crown with an above average diameter (at least one standard deviation above the mean) and with no female strobilus on the current and previous year's shoots (vigorous buds with low likelihood of female strobilus production the following year).

b) small buds - terminal buds located in the lower part of the crown with a below average diameter (at least one standard deviation below the mean) and with no female strobili on the shoots (the buds in which male strobili but not female strobili are expected the following year). Thus, four types of terminal buds are recognized in this study and are summarized in Table 2.

Ten branches for each of the four types of terminal buds from the two flowering groups were selected, and the following characteristics were recorded: past female cone production (number of first-year and second-year cones), branch order, and height from the ground. Terminal bud diameter increments were measured with a microcaliper three times during the growing season on July 26, August 23, October 25, and on February 20 the following year. On February 20, number of female or male strobili produced, shoot length of the previous growing season, and number of flushes were recorded. Total needle fresh weight per shoot was measured after harvesting the entire shoots. Relations between female or male flowering and various shoot characteristics were analyzed with simple and multipe linear regression models.

Anatomical Characteristics of Terminal Buds

Six buds (three large and three small buds) from each flowering group were harvested on July 26, August 17, and September 7. The buds were preapred for microscopic

Table 2. Summary of the morphological characteristics and flowering behavior of four types of terminal buds in abundant-flowering and poor-flowering groups of slash pine.

	flowering group			
	abundant-flowering group		poor-flowering group	
terminal bud size	large buds	small buds	large buds	small buds
flowering in previous years	female	male	vegetative	male
expected flowering in following year	female	male	vegetative	male
position in the crown	upper	lower	upper	lower

examination. First, buds were fixed in an FAA solution (a mixture of 90 ml 50% ethyl alcohol, 5 ml glacial acetic acid, and 5 ml 40% commercial formalin), dehydrated in an ethyl alcohol-tertiary butyl alcohol series (Jensen, 1962), paraffin infiltrated with Paraplast (melting point 56-57°C, by Sherwood Medical Industries), embedded, and sectioned with a rotary microtome. A series of cross sections as well as median longitudinal sections of 10 to 12 μ thick were prepared. The slides were stained with a safranin-fast green combination. Within cross sections (sectioned at the most distal lateral appendages), cambial development was classified into one of five developmental stages:

1) discrete fascicular cambium stage;
2) interfascicular cambium forming;
3) vascular cambium completed;
4) deposition of secondary xylem and phloem initiated and number of cells in secondary xylem in a radial direction was less than 25;
5) deposition of secondary xylem advanced and number of cells was greater than 25.

Diameter of phloem cells in cross section was measured with a micrometer. Also the number of cells in secondary phloem and undifferentiated phloem initials in a radial direction was counted and compared between the two flowering groups.

Relationships Between Size, Weight, and Volume of Terminal Buds

Ten terminal buds (five large and five small buds) from each flowering group were collected on September 7 and cut at the most distal lateral appendages. Their fresh weight, diameter, and length were measured. Each bud was placed in an aluminum cup and oven-dried for 24 hours at 75°C. Then dry weight of each bud was measured. The ratios of dry to fresh weight and of weight to volume were calculated.

Time of Floral Bud Initiation

Terminal buds from the upper crown of abundant-flowering trees were collected at weekly intervals from July 8 to August 13, and on September 3, 17, and October 25. The buds were prepared for microscopic examination as described previously. A series of median longitudinal sections 10 to 12 μ thick was prepared and stained with a safranin-fast green combination. Each slide was examined for the presence of floral bud primordia by light microscopy.

Results

The number of female strobili produced per tree in four selected clones of Pinus elliottii in 1976 is shown in Table 3. The abundant-flowering group (AFG) produced about five times more female cones than the poor-flowering group (PFG). The classification of these clones into the two groups based on the flowering from 1960 to 1965 (as shown in Table 1) was confirmed by this counting in 1976. Male

Table 3. Number of female strobili produced per tree in four selected clones of Pinus elliottii in 1976.

poor-flowering group		abundant-flowering group	
clone No.	No. of female strobili	clone No.	No. of female strobili
248-55	63	243-55	274
123-56	60	116-56	391

flowering was more abundant in PFG than in AFG, indicating a genetic influence on sexuality of the trees.

Shoot Vigor and Strobilus Production

The relationship between bud diameter growth and female and male flower production in abundant- and poor-flowering groups of slash pine is shown in Table 4. Diameters of both large and small buds in the abundant-flowering group (AFG) were larger in July than those of corresponding buds in the poor-flowering group (PFG). Subsequent diameter growth in August, October, and February showed no significant size difference between the two types of large buds. Male and female flowering in the two flowering groups reflected the strong genetic influence (Table 4).

Large buds in AFG produced exclusively female flowers (1.5 female strobili per bud), whereas those in PFG stayed in a vegetative state or produced male flowers (14.6 male strobili per bud). Small buds of PFG produced two times as many male flowers per bud as small buds of AFG. None of the small buds in either flowering group produced female flowers.

When a relationship between female flowering and characteristics of shoot morphology was tested by simple or multiple linear regression models, needle weight (total fresh weight of needles per shoot produced during the current growing season) was the most closely related single variable (R^2 = 0.41, statistically significant at 1% level) to the

Table 4. Bud diameter and subsequent female and male flowering in four types of terminal buds from abundant-flowering and poor-flowering groups of Pinus elliottii.

flowering group	bud size	diameter[1]				total needle weight per shoot	Average number[2] of flowers/bud	
		July	August	October	February	February	female	male
		cm				g[3]		
abundant	large	0.795A	0.817A	0.853A	0.873A	123A	1.5	0.0
poor	large	0.757B	0.789A	0.840A	0.877A	158B	0.3	14.6
abundant	small	0.633C	0.664B	0.739B	0.751B	63C	0.0	13.7
poor	small	0.607D	0.645B	0.722B	0.739B	83D	0.0	27.0

[1]Bud diameter was measured with a microcaliper in July, August, October of 1976, and February of 1977. Each number is an average of ten observations.

[2]The number of flowers produced was counted in February of 1977.

[3]The numbers within a collection date are significantly different at 5% level if they are suffixed with a different letter.

number of female flowers produced the following year (Table 5). The second most closely related variable was shoot length (R^2 = 0.37) which had a close positive correlation with needle weight (R^2 = 0.92). Bud diameter measured in August was the third most closely related variable (R^2 = 0.36). Thus, needle weight was better related to the number of female flowers than any other shoot characteristics. Two or more variables were used for a multiple linear regression. Regression was improved when all the variables were included in the model (R^2 = 0.695).

There was a negative correlation between needle weight and number of male flowers (Figure 1). The number of male flowers showed a tendency to decrease with increasing needle weight, whereas the number of female flowers increased with increasing needle weight.

Anatomical Characteristics of Terminal Buds

When cross sections of terminal buds were examined, large buds of PFG showed more advanced cambial development than large buds of AFG (Table 6). Small buds of AFG and PFG showed no significant difference in cambial development. When large and small buds within each flowering group were compared, cambial growth was more progressed in large buds than in small buds. The size (diameter) of individual cells in the phloem showed no significant differences between flowering groups or between large and small buds. However, the number of cells in secondary phloem in a radial direction

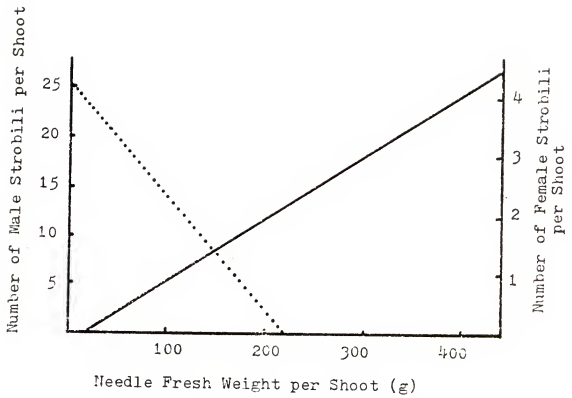
Table 5. Linear regression between some morphological characteristics of shoots and number of female or male flowers produced in Pinus elliottii.

Model	R^2
F = NF	0.052
F = BDO	0.223
F = BO	0.246
F = BDJ	0.284
F = FYC	0.290
F = BDA	0.357
F = SL	0.371
F = NW	0.405
F = NW SL	0.409
F = NW BDJ FYC	0.510
F = SL BDA FYC	0.530
F = NW BDA FYC	0.532
F = NW SL BDA FYC	0.538
F = NW BDA FYC BO NF	0.617
F = NW BDA FYC BO NF SL SYC BDJ BDO	0.695
M = NW	0.214
M = FYC	0.251

Symbol identities:

- F: number of female flowers per shoot produced in 1977
- M: number of male flowers per shoot produced in 1977
- NW: needle weight per shoot (fresh weight of needles produced in 1976 and measured in February of 1977)
- SL: shoot length (shoot growth during 1976 and measured in February of 1977)
- BDJ: bud diameter in July of 1976
- BDA: bud diameter in August of 1976
- BDO: bud diameter in October of 1976
- FYC: number of female flowers produced in 1976 (number of one-year-old cones)
- SYC: number of female flowers produced in 1975 (number of two-year-old cones)
- BO: branch order
- NF: number of flushes during the growing season of 1976

Figure 1. A relationship between needle weight (NW) and number of male (M) or female (F) flowers produced in Pinus elliottii. Needle weight refers to the total fresh weight of current-year needles per shoot produced during the growing season of 1976, and measured in February of 1977. The number of male or female strobili refers to the number of strobili per shoot produced in 1977. (n=80, p=0.01)



Line Identities:

——— : F = -0.3505 + 0.0111 NW (R^2= 0.405)

••••••••••••••• : M = 25.6639 - 0.1169 NW (R^2= 0.214)

Table 6. Characteristics of vascular tissues (in transverse section) of four types of terminal buds collected from abundant-flowering and poor-flowering groups of Pinus elliottii during the period of floral bud initiation.

flowering group	bud size	stage of cambial development[1]			average diameter of phloem cells[2]			number of phloem cells[3]		
		Jul. 26	Aug. 17	Sep. 7	Jul. 26	Aug. 17	Sep. 7	Jul. 26	Aug. 17	Sep. 7
					--------μ---------					
abundant	large	2.8A[4]	3.8A	3.8A	12.2A	11.3A	11.2A	15.8A	15.0A	16.2A
poor	large	3.4B	4.2B	4.2B	10.9A	10.7A	11.2A	17.0B	19.4B	18.8B
abundant	small	2.3C	2.8C	3.2C	11.7A	11.2A	10.9A	15.0A	15.2A	15.4A
poor	small	2.0C	2.8C	3.0C	11.4A	10.9A	10.7A	14.3A	16.6A	15.2A

[1]Stage of cambial development in transverse section sectioned at the most distal lateral appendages (each number is an average of six discrete numbers from 1-5 as shown below).
1. discrete fascicular cambium stage;
2. interfascicular cambium forming;
3. vascular cambium completed;
4. deposition of secondary xylem and phloem under way (number of cells in secondary xylem in a radial direction less than 25);
5. deposition of seconeary xylem advanced (number of cells greater than 25);

[2]Average diameter (in micron) of phloem cells in transverse section.

[3]Number of cells in secondary phloem and undifferentiated phloem initials in a radial direction.

[4]The numbers within a collection date are significantly different (at 5% level) if they are suffixed with a different letter.

was greater in large buds of PFG than in those of AFG. Thus, large buds of PFG had a significantly greater number of secondary xylem and secondary phloem than large buds of AFG.

Median longitudinal sections of terminal buds showed that the number of cataphylls in the fertile series (subtending the bud primorida) in large buds was much greater than in small buds (Figure 2). Apical meristems of large buds of both flowering groups were much larger and wider than those of small buds of either flowering group.

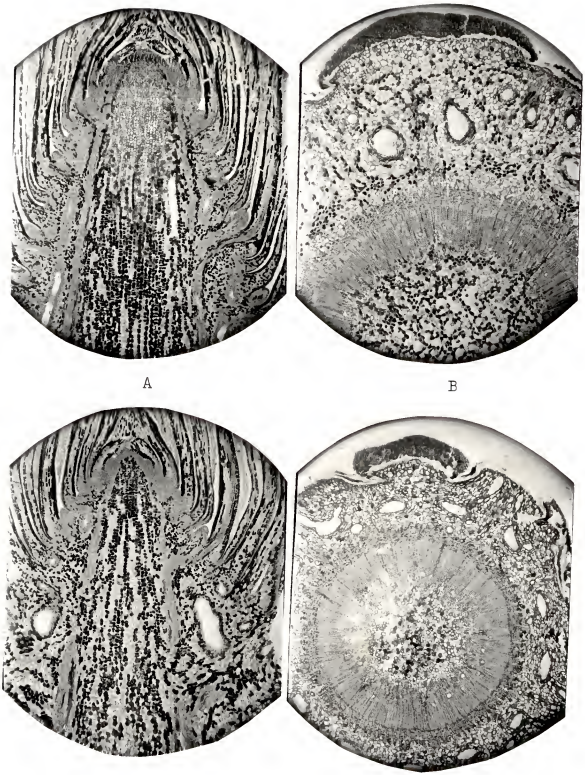
Figure 2. Median longitudinal and cross sections of large and small buds of the abundant-flowering group of Pinus elliottii collected on July 26.

A) A median longitudinal section of a large bud (mag. 22x)

B) A cross section of a large bud (mag. 22x)

C) A median longitudinal section of a small bud (mag. 22x)

D) A cross section of a small bud (mag. 22x)



A B C D

Relationships Between Size, Weight, and Volume of Terminal Bud

Within a tree, volume, fresh and dry weights of large buds were about three times greater than those of small buds (Table 7). The ratio of dry weight to fresh weight showed little difference between large and small buds of the same tree, and the ratio appeared to be a characteristic of individual trees (column E in Table 7). When dry weight was expressed as weight per unit volume, no difference was observed between large and small buds of AFG, whereas a significant difference was noticed between large and small buds of PFG. This indicated that small buds of PFG had a more compact structure than large buds of the same group.

Table 7. Relationships between dry weight, fresh weight, and volume of terminal buds of abundant-flowering and poor-flowering groups of slash pine collected on September 7.

			A	B	C	D	E	F
flowering group	clone no.	bud size	diameter (cm)	volume (cm^3)	fresh wt. (g)	dry wt. (g)	dry wt./ fresh wt.	dry wt./ volume
abundant	243-55	large	0.717[1]	0.349[2]	0.952	0.419	0.440A	1.20A
abundant		small	0.480	0.103	0.288	0.127	0.440A	1.24A
abundant	116-56	large	0.684	0.298	0.774	0.330	0.429A	1.14A
abundant		small	0.514	0.098	0.263	0.111	0.421A	1.15A
poor	123-56	large	0.718	0.567	1.627	0.625	0.382A	1.22A
poor		small	0.456	0.123	0.482	0.179	0.372A	1.48B
poor	248-55	large	0.694	0.338	0.771	0.340	0.441A	1.00A
poor		small	0.508	0.121	0.332	0.146	0.439A	1.21F

[1]Each number is an average of 5 observations.

[2]To calculate the volume, each bud was assumed to have a conical shape.

In columns E and F, two numbers within a clone are significantly different at 5% level if they are suffixed with a different letter.

Time of Floral Bud Initiation

In the buds collected on July 8 no evidence of primordia of staminate strobili was noticed. A bud collected on July 16 showed a distinct primordium of a staminate strobilus in the lowest portion of the fertile cataphyll series (Figure 3A). Also in some axils of cataphylls distinct prominences were observed. By July 30 male strobilus primorida were visible in the lower part of the fertile series (Figure 3B). The lower meristematic area started to differentiate hood scales, and by August 6 several hood scales were formed (Figure 3C). Vascular differentiation on male primordia was distinctly visible by August 13 (Figure 3D). Initiation of rudimentary microsporophylls was visible on September 3 (Figure 3E) and was more pronounced on September 17 (Figure 3F).

The primordia for the female strobili were not noticed until September 17. They appeared near the bud apex as protuberances, with distinctive, heavy staining of cell inclusions (Figure 3G). Subsequent development of female strobili was not followed in this study.

Figure 3. Development of staminate and ovulate strobilus primordia of Pinus elliottii collected from a seed orchard located in Gainesville, Florida.

A) Median longitudinal section of a terminal bud collected on July 16 (mag. 22x)

B) Primordia of staminate strobili in the axils of expanding cataphylls by July 30 (mag. 22x)

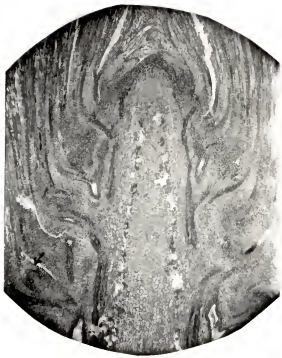
C) Several hood scales were formed by August 6 (mag. 55x)

D) Vascular differentiation was visible on August 13 (mag. 55x)

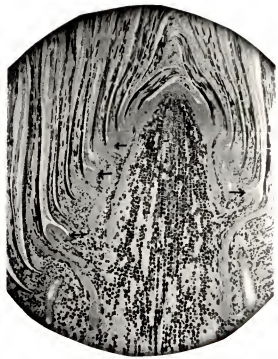
E) A few rudimentary microsporophylls were formed by September 3 (mag. 22x)

F) Rudimentary microsporophylls on September 17 (mag. 22x)

G) A primordium of an ovulate strobilus was shown near the bud apex as a slight protuberance on September 17 (mag. 55x)



A



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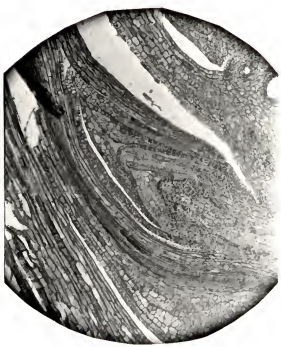
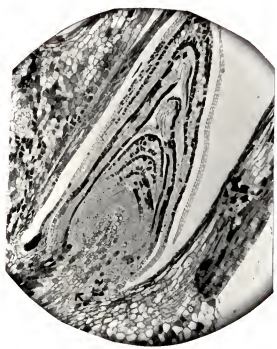
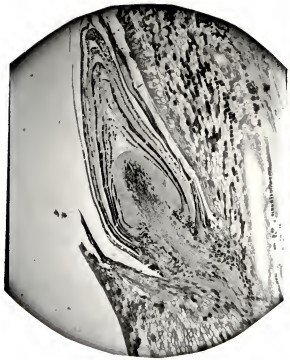


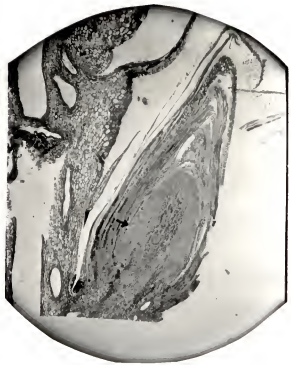
C



D



E



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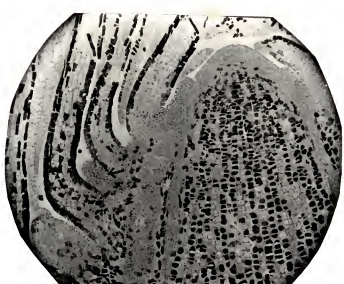


G

Discussion

A strong genetic influence on female and male flowering in Pinus elliottii was observed in this study. The number of female and male strobili produced per tree or per shoot in the two flowering groups strongly suggested that the abundant-flowering group (AFG) was predominantly female-producing, whereas the poor-flowering group (PFG) was predominantly male-producing. The mechanism of sex determination in pines is not fully understood. Potential of trees to produce female cones appears to be biologically fixed and controlled by unknown internal physiological conditions.

The relationship between shoot morphology and flowering indicated that needle weight (current-year needles) was the single variable most closely related to the number of female flowers produced the following year on the same shoot. Length of current-year shoots was the second best indicator for future cone production. This agrees with the study of Varnell (1970) who noticed that cone-bearing secondary branches in Pinus elliottii had longer and thicker shoots and terminal buds than nonbearing branches. He showed that diameter was more closely associated with production of female strobili than shoot length, and was probably due to a more direct reflection of current environmental conditions on radial growth than on extension growth.

It should be mentioned that the high correlation (R^2 = 0.41) between total needle weight per shoot and number of

female strobili produced per shoot might result from an intentional selection of large and small buds in the upper and lower parts of crown, respectively. This biased selection of the two different types of buds (rather than a random selection around the crown) might result in a forced correlation between needle weight and number of female strobili produced. When only the large buds of both flowering groups (n = 40) were used for the linear regression (model: F = NW as shown in Figure 1), the correlation decreased considerably (R^2 = 0.16). Thus, shoot vigor alone does not distinguish between abundant-flowering and poor-flowering trees.

The significance of needle weight suggested that needles on a given shoot might contribute, in some way, to the initiation of floral bud primordia. The influence of current-year needles appeared to be qualitatively as well as quantitatively associated with flowering as judged by the tendency for transition from male to female flowering (a qualitative change) to be associated with increasing vigor of the shoot, and judged by the significant correlation of the number of female flowers (a quantitative measurement) with needle weight. Possibly the current-year needles either produce chemical stimuli that are transported to the terminal bud or simply improve carbohydrate nutrition of the terminal bud.

Relationships between size, weight, and volume of the terminal buds of the two flowering groups indicated that dry weight per unit of volume was significantly less in the large buds of PFG than in the small buds of the same group,

whereas no difference was observed between the two types of buds in the AFG. This suggested that the large buds of PFG, which most likely stayed in a vegetative state, maintained a less compact structure than the small buds of the same group. The less compact structure seemed to result from the active vegetative growth in the large buds of PFG.

When cross sections of terminal buds were examined under a light microscope, the size of individual cells in phloem tissue showed no difference between large and small buds. But a difference was observed between the large and small buds in the stage of cambial development and subsequent deposit of secondary xylem and phloem. It is likely that the greater number of secondary xylem and phloem cells in the large buds of PFG than in other three types of buds might enhance transport of nutrients and carbohydrates into the buds, which in turn could favor further vegetative growth.

Results of the study of the timing of floral bud initiation were close to those given by Mergen and Koerting (1957). The first indications of male and female strobilus primordia in the present study were observed on July 16 and September 17, respectively, whereas Mergen and Koerting (1957) reported the time of initiation to be the end of June and late August, respectively. The discrepancy may reflect two possible reasons. First, it was difficult to distinguish between short-shoot and male flower primordia at early stages of development. Second, year to year variation in climatic conditions cause fluctuation in the time of floral bud

initiation. Doak (1935) presented an average period of floral bud initiation for Pinus; male flower primordia are initiated near the end of July and female flower primordia are initiated during the middle of August. His statement appeared to agree with flower initiation in Pinus elliottii.

CHAPTER IV
CARBOHYDRATE AND NITROGEN METABOLISM OF TERMINAL BUD IN RELATION TO STROBILUS INITIATION

Introduction

Kramer and Kozlowski (1960) stated that a high level of carbohydrates was required for flower initiation in woody plants. More specifically, it has been proposed that a high carbohydrate to nitrogen (C/N) ratio promotes flowering (Kraus, 1925).

In conifers the amount of light received by individual trees seems to be related to their capacity to produce cones (Fowells and Schubert, 1956). Cultural treatments which increase light interception, such as thinning, release, and branch pruning are believed to stimulate flowering primarily through enhanced photosynthesis and subsequent accumulation of carbohydrates. The requirement of sunny days during the early growing season for abundant flowering the following year (Shoulders, 1973) also supports the positive role of carbohydrates in flower initiation.

Factors related to the uptake of mineral nutrients, such as site fertility, fertilization, and irrigation have been shown to influence flowering in conifers. Particularly nitrogen fertilization has been used successfully to stimulate female flowering (Barnes and Bengtson, 1968; Schmidtling,

1974). The stimulating effect of nitrogen fertilization seems to be one of the experimental observations contradicting the C/N theory.

Experiments were undertaken to understand the physiological conditions of terminal buds at the time of floral bud initiation in relation to the pool levels of extractable carbohydrates and amino acids in the terminal buds.

Materials and Methods

Four clones, two from the abundant- and two from the poor-flowering group of slash pine (Pinus elliottii var. elliottii Engelm.), were used in this study. Four types of terminal buds in the two flowering groups as described in the preceding chapter (Table 2) were recognized. Buds were collected on July 28, August 4, August 26, and September 2. The buds were cut and frozen in liquid nitrogen in the field. The frozen buds were brought to the laboratory and ground with a mortar and pestle to fine powder. The ground bud tissue was freeze-dried and stored in a freezer for further analyses.

Extraction of Free Sugars and Free Amino Acids

About 0.5 g of freeze-dried bud tissue was extracted with 25 ml of 75% ethanol for 20 min. at 70°C and filtered. The residue was extracted again with 25 ml of fresh 75% ethanol at 70°C for 1 hour and the extract was filtered. The extraction was repeated once more for 1 hour using the

same amount of fresh ethanol. The extracts were combined in a flask and concentrated to about 15 ml in a rotary evaporator at 35°C to remove the alcohol. The concentrate was transferred to a separatory funnel, and the pH of the solution was adjusted to 3.0 with 0.01 N HCl. Twenty ml of hexane were added and the concentrate was partitioned into an aqueous and a hexane fraction. The hexane fraction was discarded and the aqueous fraction was partitioned with hexane two more times. The resulting aqueous fraction was centrifuged for 20 min. at 27,000x g. The supernatant fraction was subjected to cation exchange chromatography.

Cation Exchange Chromatography

Dowex 50W-X8 resin (hydrogen form, 200-400 mesh) was used as described by Splittstoesser (1969). The commercial resin was suspended in two volumes of distilled water, shaken and allowed to stand until the majority of the resin beads settled. The upper liquid was decanted to remove fine resin particles. The resin was poured into a 1.2 cm (internal diameter) by 15 cm long glass column as a thick slurry and was packed with air pressure. Care was taken to prevent air bubbles from being trapped in the resin column. The resin was washed first with 75 ml water and the eluate was discarded. Then the bud extract (supernatant of step "M" in Figure 4) followed by 25 ml water were run through the column and the eluate (an acidic fraction) was saved. The column was washed with 50 ml 0.4 N NH_4OH, 25 ml water,

25 ml 4 N NH_4OH, and finally 25 ml water in that order. The eluate (a basic fraction) was saved. Liquid level was maintained above the top of the resin at all times to prevent penetration of air through the resin. The elution rate was kept at about 4 ml per minute under slight air pressure.

Anion Exchange Chromatography

The method described by Splittstoesser (1969) was used here. Dowex 1-X8 (formate form, 200-400 mesh) was prepared in a glass column the same as for the cation exchange chromatography. The acidic fraction (step "P" in Figure 4) was brought to a final volume of 100 ml, and a 50 ml aliquot was pipetted into a beaker. The solution was adjusted to pH 8.0 with 1 N and 0.1 N NH_4OH solutions, and eluted through the resin. The eluate was saved. The resin was washed with 25 ml water, and the eluate was combined with the previous one. This combined eluate (a neutral fraction) contained sugars (step "V" in Figure 4). The resin which adsorbed organic acids was washed with 50 ml 8 N formic acid and with 25 ml water. This eluate contained organic acids (step "W" in Figure 4).

Gas Chromatography of Sugars

The neutral fraction after anion exchange chromatography (step "V" in Figure 4) was used for gas liquid chromatography of sugars. Methods described by Ford (1974) and Fretz et al. (1970) were used.

Instrumentation. The apparatus used was Packard Becker Gas Chromatograph Model 421 equipped with dual hydrogen-flame ionization detectors. Separation of sugars was performed with a Pyrex glass column (U-shape, 0.64 cm O.D. x 182 cm) packed with 3% SE-30 (G.C. grade) on Chromosorb WHP (80-100 mesh, obtained from Applied Science Laboratories, Inc.).

Operating Conditions. Injection port and detector temperatures were maintained at 275°C. Column temperature was initially 125°C for 5 min., programmed to rise linearly at 4°C/min. to 260°C, and held at 260°C for 10 min. Separation of L-arabinose, L-rhamnose, D-ribose and L-fucose was improved by maintaining the initial temperature at 125°C for 5 min., since these sugars separated poorly if initial temperature was maintained higher than 125°C or if the initial temperature was maintained at 125°C less than 5 min. Temperature rise programmed at 4°C/min. gave a better separation of both pentoses and hexoses than programmed at 5°C/min. or higher. A 3 μl sample was injected into "A" column, while "B" column served for compensation of base line drift during temperature programming. Nitrogen was used as a carrier gas at a flow rate of 25 ml/min. Hydrogen and air flow rates were 25 ml/min. and 250 ml/min., respectively. A new column was conditioned at 280°C for 24 hours before use. A strip chart recorder was used at chart speed of 1.3 cm/min.

Preparation of Sugar Standards. The sugar standards used here include L-arabinose, D-xylose, D-ribose, D-glucose, D-fructose, D-mannose, D-galactose, L-rhamnose, L-fucose, and sucrose. The sugar alcohol standards were xylitol, adonitol, mannitol, D-sorbitol, and pinitol. Twenty milligrams of each sugar were dissolved in 20 ml anhydrous pyridine, and 0.5 ml of the sugar solution was mixed with 0.1 ml 1-(trimethylsilyl)-imidazole (purchased from Eastman Kodak Co.) in a glass vial, and left at room temperature for 10 min. One microliter aliquot of the silylated sugar solution was injected with a microsyringe into the gas chromatograph.

Sample Preparation. The neutral fraction (step "V" in Figure 4) was made to a volume of 100 ml. Twenty ml aliquot was pipetted into a boiling flask, dried completely under vacuum at 35°C, redissolved with 2.0 ml anhydrous pyridine. An 0.5 ml aliquot was pipetted into a small glass vial and mixed well with 0.1 ml 1-(trimethylsilyl)-imidazole. A stopper made of rubber should be avoided while mixing and shaking above mixture. After 10 min. 3 µl aliquot of the silylated solution was injected into the gas chromatograph. Each sample was chromatographed in triplicate.

Identification and Quantification of Peaks. Two anomer (α and β) peaks were observed in D-xylose, D-mannose, D-fructose, D-galactose, and D-glucose. It was shown that during chromatography α- and β-anomers of mannose maintained a ratio of 4:1. Fructose anomers (α and β) showed a ratio

of 1:1. Thus, it was possible to calculate the contribution of mannose to the total peak when both mannose and fructose were present in the sample. Peaks in the sample chromatogram were identified by comparing retention times with those of known standard sugars. An individual sugar was quantified by using a ratio of sample peak area (area under the peak) to that of corresponding standard sugar. Total sugar content was expressed in mg/g dry weight.

Determination of Free Amino Acids

The basic fraction (step "Q" in Figure 4) which contained amino acids was dried completely under vacuum, redissolved with 10 ml of 0.01 N HCl, and filtered with a millipore filter (pore size 0.2 μ). The filtrate was analyzed for amino acids using an amino acid analyzer (Japan Electron Optics Laboratory Co., Model JLC-6AH). A standard amino acid solution (commercially made) containing 100 n moles/ml of each amino acid commonly found in proteins was used to quantify individual amino acids. The following formula was used for each amino acid:

$$\frac{\text{peak area (sample)}}{\text{peak area (standard)}} \times 100 \text{ n moles/ml} \times \frac{\text{sample volume (ml)}}{\text{sample weight (g)}}$$

= n moles/g.

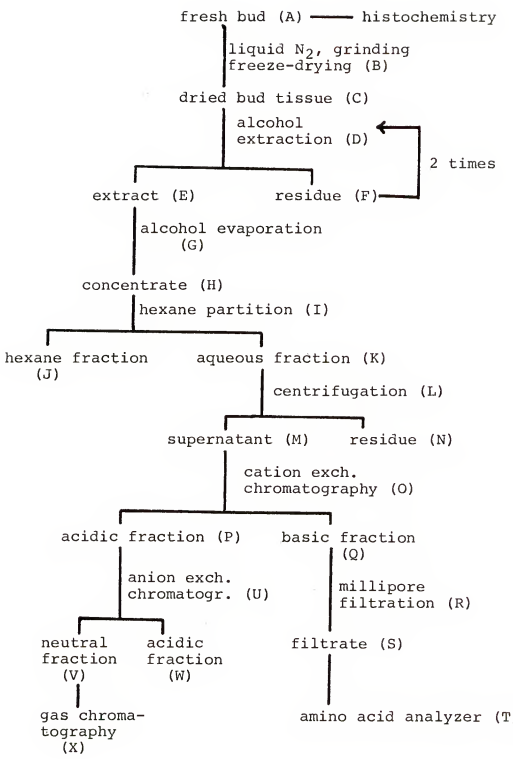


Figure 4. Extraction procedures for free sugars and free amino acids in the bud tissue of Pinus elliottii.

Results

Sugar Content in the Terminal Buds

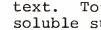
Sugar contents in the four types of terminal buds are shown in Figure 5. Glucose and fructose accounted for about half of the total sugar concentration in all four types of buds. A high amount of pinitol was detected in all the samples, and it accounted for 15 to 21% of the total sugars.

Total sugar concentration (a sum of individual sugars identified) remained relatively stable during the sampling period in all the four types of terminal buds. When total sugar concentration was compared within a flowering group, large buds of both flowering groups had a greater amount of total sugars than small buds of the same group (statistically significant at 5% level). When total sugar concentration was compared between the two flowering groups, no statistically significant difference was noticed between the female-producing (large buds of abundant-flowering group) and vegetative buds (large buds of poor-flowering group).

All the individual sugars, with the exception of mannose, fluctuated considerably. Mannitol and glucose concentration decreased gradually over the sampling period. No single sugar concentration was significantly related to the flowering potential of the buds.

Figure 5. Free (ethanol-soluble) sugar contents (mg/g dry weight) in four types of terminal buds collected in 1976 from abundant- and poor-flowering groups of Pinus elliottii. Sugars were determined by gas-chromatography as described in the text. Total sugars mean a sum of individual soluble sugars. Classifications of terminal buds and flowering groups are explained in Chapter III (Table 2). Each point is an average of two observations. Each observation was triplicated for the gas chromatographic analysis.

Line identities: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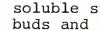
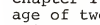
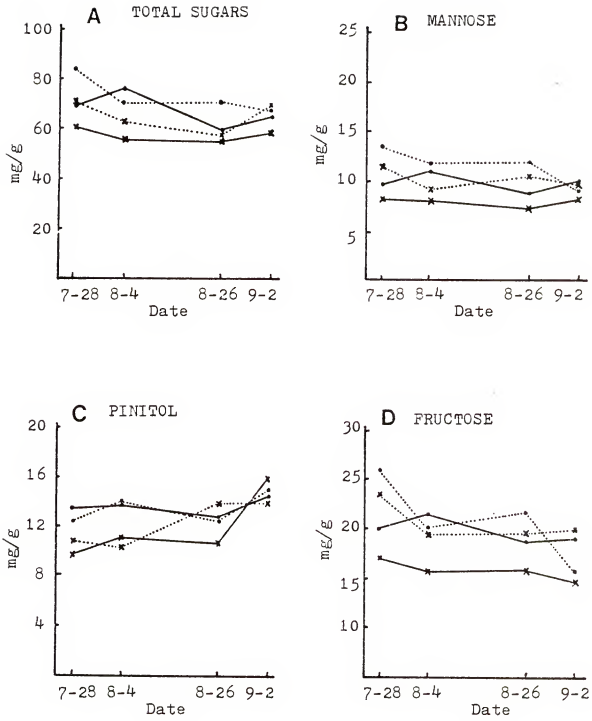
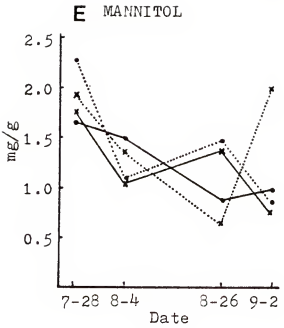
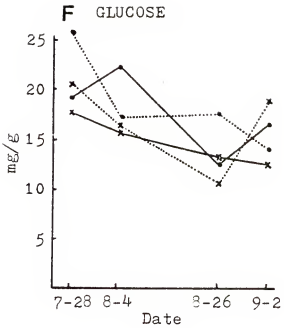

: abundant flowering group, small bud

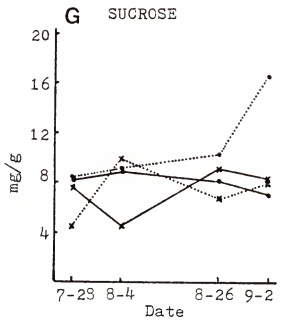

: poor-flowering group, large bud


: poor-flowering group, small bud


A
TOTAL SUGARS
100
80
60
40
20
mg/g
7-28
8-4
8-26
9-2
Date
B
MANNOSE
25
20
15
10
5
mg/g
7-28
8-4
8-26
9-2
Date
C
PINITOL
20
16
12
8
4
mg/g
7-28
8-4
8-26
9-2
Date
D
FRUCTOSE
30
25
20
15
10
mg/g
7-28
8-4
8-26
9-2
Date


E MANNITOL
2.5
2.0
1.5
1.0
0.5
mE/g
7-28 8-4 8-26 9-2
Date


F GLUCOSE
25
20
15
10
5
mE/g
7-28 8-4 8-26 9-2
Date


G SUCROSE
20
16
12
8
4
mE/g
7-28 8-4 8-26 9-2
Date

Amino Acid Content in the Terminal Buds

Seventeen different amino acids commonly found in plant proteins were detected in Pinus elliottii buds. An amino acid which eluted between serine and glutamic acid was found in most of the samples but was not identified.

Total and individual amino acid contents in the samples are shown in Figure 6. The following amino acids, histidine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine were detected in small amounts (less than 0.2 μ mole/g), and were not included in Figure 6. However, the quantity of histidine, cysteine, valine, isoleucine, and leucine were included in the calculation of the total amino acids.

Arginine was the most abundant amino acid in all four types of buds, and its relative concentration to total amino acids increased with time from 23.3% of total on July 28 to 29.0% on August 4, 47.9% on August 26, and 60.2% on September 2, indicating the accumulation of arginine in all four types of buds. Individual amino acids varied considerably during the observation period. Certain changes were noticed in some amino acids: Basic (positively charged) amino acids, lysine and arginine, varied with a similar pattern (increasing over the sampling period), while acidic (negatively charged) amino acids, aspartic acid and glutamic acid shared another common pattern (suddenly decreasing at the end of the sampling period).

Total amino acid content in the buds (Figure 6A) increased considerably during the sampling period. An exception was observed in the large buds of the abundant-flowering group (AFG) where the concentration of total amino acids remained virtually the same during the sampling period and were lower than the other three types of buds in the last three sampling dates. When large and small buds of AFG were compared, small buds contained about four times more total amino acids than large buds at the end of the sampling period (on September 2).

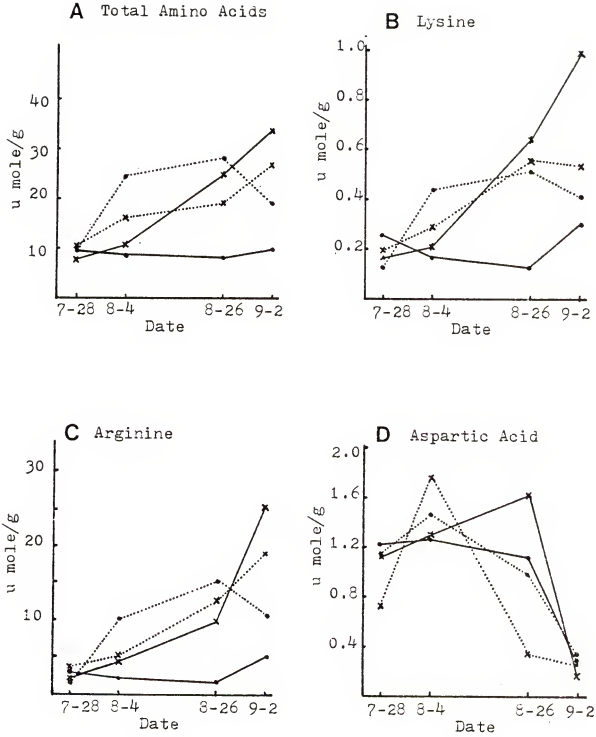
Lysine and arginine concentration in the large buds of AFG was significantly lower than in any of the other three types of the terminal buds during the last two sampling dates (on August 26 and September 2). The low total amino acid concentration in the large buds of AFG resulted mainly from the low concentration of arginine in the bud tissue.

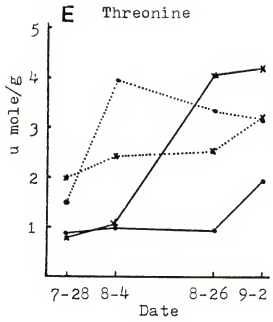
Sugar to Amino Acid Ratio in the Bud

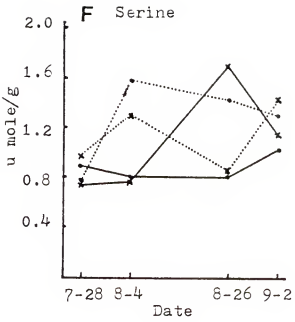
The amount of free sugars relative to amino acid content (the ratio of sugars to amino acids) is shown in Figure 7. The ratio in the large buds of AFG was significantly higher than in the other three types of buds during the last three sampling dates (August 4 and 26, and September 2). In general, the ratio in the four types of buds decreased with time due to increased concentration of amino acids during the last three sampling dates.

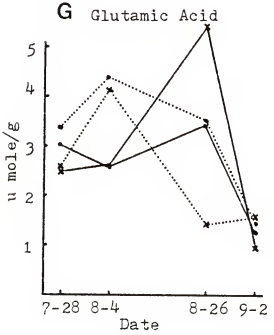
Figure 6. Free (ethanol-soluble) amino acid contents in four types of terminal buds collected in 1976 from abundant-flowering and poor-flowering groups of Pinus elliottii. Amino acids were determined by an automatic amino acid analyzer as described in the text. Total amino acids mean a sum of individual amino acids in μ moles/g of dry weight. Classifications of terminal buds and flowering groups are explained in Table 2 of Chapter III.

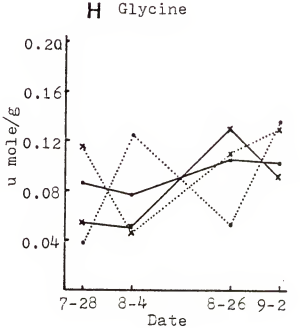
Line identities:

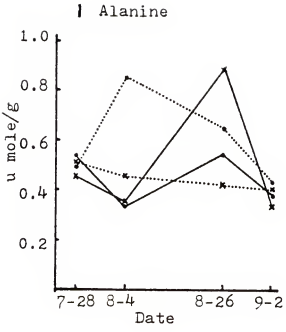

A Total Amino Acids
u mole/g
40
30
20
10
7-28 8-4 8-26 9-2
Date
B Lysine
u mole/g
1.0
0.8
0.6
0.4
0.2
7-28 8-4 8-26 9-2
Date
C Arginine
u mole/g
30
20
10
7-28 8-4 8-26 9-2
Date
D Aspartic Acid
u mole/g
2.0
1.6
1.2
0.8
0.4
7-28 8-4 8-26 9-2
Date


E Threonine
u mole/g
5
4
3
2
1
7-28 8-4 8-26 9-2
Date


F Serine
u mole/g
2.0
1.6
1.2
0.8
0.4
7-28 8-4 8-26 9-2
Date


G Glutamic Acid
u mole/g
5
4
3
2
1
7-28 8-4 8-26 9-2
Date


H Glycine
u mole/g
0.20
0.16
0.12
0.08
0.04
7-28 8-4 8-26 9-2
Date


Alanine
1.0
0.8
0.6
0.4
0.2
u mole/g
7-28
8-4
8-26
9-2
Date

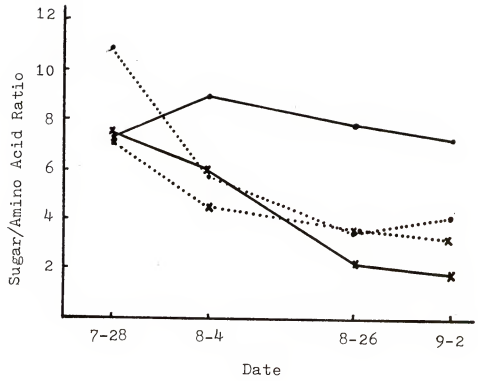


Figure 7: Ratio of free sugars (ethanol-soluble) to free amino acids (ethanol-soluble) in the four types of the terminal buds from abundant-flowering and poor-flowering groups of Pinus elliottii during the period of female flower initiation. Sugars were determined by gas-liquid chromatography and amino acids by an automatic amino acid analyzer. Classifications of terminal buds and flowering groups are explained in Chapter III (Table 2). Each point is an average of two observations from two different trees.

Line identities:

●——●——●: abundant-flowering group, large bud
×——×——×: abundant-flowering group, small bud
●·····●·····●: poor-flowering group, large bud
×·····×·····×: poor-flowering group, small bud

Discussion

Analysis of free sugars in the terminal buds showed that the large buds of both flowering groups had greater amounts of soluble sugars than the small buds of the same group. This indicates that large buds (surrounded by large numbers of needles than small buds as shown in Table 4 of Chapter III) received greater amounts of carbohydrates from the surrounding needles than small buds. This might explain morphological characterisitcs of the large buds which might require larger supplies of carbohydrates than the small buds for the structural growth. It was difficult, however, from free sugar data alone to characterize the specific condition of the large buds of abundant-flowering group (AFG) during the period of female flower initiation, because no difference was observed in free sugar content between the large buds of AFG and those of poor-flowering group (PFG) during the period of observation.

Results of the amino acid analysis suggests that smaller amounts of amino acids were available to the large buds of AFG than to other three types of buds studied. When individual amino acids were compared, arginine content in the large buds of AFG was lowest among the four types of buds.

The lower level of arginine in the large buds of AFG (where female flowers are expected to be formed) raised a doubt about a positive role for arginine in female flower initiation. Barnes and Bengtson (1968) observed that when

nitrogen fertilizer was applied to Pinus elliottii, female flowering was doubled and total free amino acids, especially arginine, increased in needles and twigs. Ebell and McMullan (1970) noticed large accumulations of arginine after Pseudotsuga menziesii were treated with nitrogen fertilizer, which also increased female cone production by seven-fold. They proposed that this arginine-type metabolism was quantitatively associated with female cone production. The present study, however, indicates that arginine is not accumulated in the bud, the site of initiation, of abundant-flowering trees. It is likely that accumulation of arginine in their studies may simply reflect the abundance of nitrogenous compounds as a storage form of excess nitrogen after heavy fertilization. Accumulation of arginine appears to be quite common in pine species. For example, Ebell (1969) reported that accumulation of arginine during period of water-stress, and Durzan and Steward (1967) observed the accumulation of arginine in Pinus banksiana seedlings when they were grown under favorable mineral nutrient conditions.

A study similar to the present experiments was reported by Stanley and Smith (1970). They analyzed nitrogen content, diffusible ions, and protein amino acids in needles and buds of Pinus elliottii from "high" and "low" flowering trees following nitrogen fertilization. Total nitrogen of the high flowering trees before and after fertilization was significantly higher than that of the low flowering trees. Total diffusible ions showed that the low flowering

trees had significantly higher diffusible ions than the high flowering trees. They suggested that nitrogen in the high flowering trees was incorporated into more stable forms than in the low flowering trees. But they did not suggest involvement of specific metabolites in the floral induction process.

Kozlowski (1971) suggested that a higher nutritional status is required for development of female cones than for male cones. He did not specifically explain what "a high nutritional status" means except a situation associated with accumulation of carbohydrates. It is likely that the relative availability of carbohydrates to nitrogen (more specifically amino acids), rather than absolute amounts of carbohydrates alone, might be a better indication of the nutritional status of the tree.

When a free sugar to amino acid ratio was compared in the present study, large buds of AFG showed higher values than the other three types of buds during the period of female flower initiation. This trend was primarily due to the low amino acid content of the female-producing buds (the large buds of AFG). This indicated that female flower initiation was associated with a relatively low supply of nitrogen during the time when the supply of carbohydrates was not limiting. This might lead to "a nutritional stress" which is responsible for the transition of the meristem to a reproductive phase.

Since a high C/N ratio was first proposed to promote flowering in herbaceous plants by Kraus and Kraybill (Kraus, 1925), it was also reported to be associated with flowering in some conifers. For example, flowering of nitrogen-starved Cupressus arizonica (Kuo, 1973; Kamienska et al., 1973) and Cryptomeria japonica (Lyr and Hoffmann, 1964) and gibberellin-treated (Hashizume, 1961) and girdled trees of the latter species (Hashizume, 1970) was associated with a high C/N ratio.

There is evidence supporting the view that disturbance of nutritional balance may lead to a "physiological stress" and subsequent flowering. Various internal and external conditions can induce a physiological stress in trees. For example, "a specific chemical stimulation from critically timed changes in type of nitrogen metabolism" (Ebell, 1972, p. 317) after nitrogen fertilization may be due to a sudden stress or shock associated with unbalanced nutrition in the buds, rather than due to gradually improved nitrogen nutrition after fertilization. Moisture stress in summer which increased female flowering (Shoulders, 1973) may stimulate flowering through a physiological stress associated with high levels of carbohydrates and low nitrogen supply. Stimulation of flowering by girdling was related to accumulation of carbohydrates above the girdle (Ebell, 1971), but it may be explained by disturbed nutrition associated with a high C/N ratio.

Kuo (1973) and Pharis (1976) reported that induced nitrogen deficiency or nitrate fertilization increased endogenous levels of less-polar gibberellins (GAs), although the above two treatments might have opposing effects on nitrogen nutrition. It appears that GAs may play a role in measuring the balance between carbohydrates and nitrogen by means of interconversions of GAs. Increased levels of less-polar GAs in water-stressed or girdled trees (Pharis, 1976) also support this idea.

Another possible explanation for the low amino acid contents in the large buds of AFG (where female strobili are expected to be formed) may be due to termination of elongation growth accompanied by a general reduction in the capacity of these buds to act as metabolic sinks. Apical meristem activity generally declines as floral organs are initiated (Lang, 1965; Romberger and Gregory, 1974; Greenwood, 1978).

CHAPTER V
TRANSLOCATION AND DISTRIBUTION OF ^{14}C-LABELED PHOTOSYNTHATE IN TERMINAL BUDS

Introduction

Initiation and subsequent development of floral structures in trees requires mobilization of carbohydrates from photosynthesizing leaves (Zimmermann, 1967). Dickmann and Kozlowski (1968, 1970) showed that one-year-old needles (those expanded during the previous year) of Pinus resinosa were efficient exporters of ^{14}C-labeled photosynthate to the currently expanding shoots and cones. They also showed that second-year cones were stronger sinks for assimilated ^{14}C than other parts of the shoot. Translocation of ^{14}C-labeled photosynthate has been studied in young pine trees (Ursino et al., 1968; Rangnekar and Forward, 1969; Ziemer, 1971). However, little is known about translocation and subsequent metabolism of photosynthate from needles to terminal buds of adult trees during the period of floral bud initiation.

An examination of carbohydrate concentration in the terminal buds of Pinus elliottii, as described in the previous chapter, showed that large buds (female-producing) of abundant-flowering trees had higher concentration than small buds (male flower producing) of the same trees during the

period of floral primordia initiation. This suggested that female strobilus initiation took place under the influence of an abundance of carbohydrates.

The present study was initiated to understand translocation of ^{14}C-labeled photosynthate from needles to terminal buds and distribution of radioactivity in various metabolic intermediates, and their possible relationship to the timing of floral initiation, or number of flowers produced.

Materials and Methods

1975-Experiments

Four clones of slash pine (Pinus elliottii var. elliottii Engelm.) with a history of abundant female cone production (abundant-flowering group, AFG) and four clones of poor cone production (poor-flowering group, PFG) were used in this experiment (see Table 1 in Chapter III). In the AFG, branches with large terminal buds (bud diameter of at least one standard deviation above the tree mean) and with one or more current-year female strobili were selected from the upper half of the crown. In the PFG, branches with small terminal buds (bud diameter of at least one standard deviation below the tree mean) and with no current- or previous-year female strobili were selected from the lower half of the crown. Care was taken to choose branches receiving full sunlight at the time of exposure to $^{14}CO_2$.

Preparation of $Na_2{}^{14}CO_3$ Solution. Sodium carbonate ($Na_2{}^{14}CO_3$) solution, which had a specific activity of 59 mCi/mmol and a radioactive concentration of 5.0 mCi/ml of aqueous solution, was diluted 10 times with phosphate buffer (Na_2HPO_4 and KH_2PO_4 at pH 8.0) to give a radioactive concentration of 0.5 μCi/μl.

Exposure to $^{14}CO_2$. A method described by Dickmann and Kozlowski (1968) was used. A small glass vial was tied to a shoot in vertical position. The shoot was then enclosed in a heavy polyethylene bag and the open end of the bag was firmly tied around the base of the shoot. Three ml of 2 M lactic acid was injected into the vial with a syringe, and 100 μl of sodium carbonate solution (50 μCi in total radioactivity) was carefully injected into the same vial with a microsyringe. After 90 minutes the polyethylene bag was removed. The above procedures were carried out during the morning hours to avoid heat injury.

Six branches from each tree were exposed to $^{14}CO_2$ at a given treatment period. Three branches were harvested 24 hours after the treatment, and the remaining three branches were harvested seven days after treatment. Radioactive CO_2 was applied five times during the summer period on the following dates: July 22, July 31, August 12, August 21, and September 2.

Determination of Radioactivity. Each harvested shoot was divided into three parts: needles, stem, and terminal bud. Each part was freeze-dried and ground. About 100 mg

of duplicated sample was combusted by a sample oxidizer (Intertechnique IN 4101 L.S.). Radioactivity was determined by liquid scintillation spectrometry.

Methods of extraction of free sugars and free amino acids from the buds were described in the previous chapter and illustrated in Figure 8. The neutral fraction (sugars) after anion exchange chromatography was completely dried under vacuum and redissolved in a 5.0 ml water. One milliliter aliquot was pipetted into a scintillation vial and 10 ml of Aquasol-2 (New England Nuclear) was added. The basic fraction (amino acids) after cation exchange chromatography was completely dried under vacuum, redissolved with 10.0 ml of 0.01 N HCl, filtered through millipore (0.2 μ) filter. One ml of filtrate was combined with 10 ml Aquasol-2 in a scintillation vial. Radioactivity of the solution was determined by liquid scintillation spectrometry.

Radioactivity left in the bud tissue after alcohol extraction (ethanol-insoluble fraction) was determined following combustion of the tissue.

Efficiency of Separation and Recovery of Sugars and Amino Acids. Efficiency of separation and recovery of free sugars and amino acids during the extraction and ion exchange chromatography were tested by adding known amounts of radioactive sucrose (56,000 CPM) and arginine (63,000 CPM) to unlabeled pine bud tissue. The tissue was extracted with 75% alcohol for free sugars and amino acids as described in Figure 8. Recovery of radioactivity after cation exchange chromatography was measured.

1976-Experiments

Experiments conducted in 1976 recognized four different types of terminal buds as described in Chapter III (Table 2). The methods of shoot selection, exposure to $^{14}CO_2$, and harvesting were the same as those for the previous year. Radioactive $^{14}CO_2$ was applied on July 28 and August 26. The selection of above dates for ^{14}C-exposure was based on the results of previous year (1975-experiments). Six branches each for four types of buds from AFG and PFG of Pinus elliottii were exposed to $^{14}CO_2$. Three branches were harvested 24 hours after the exposure, and the remaining three branches seven days after the exposure.

Free sugars (step "V"), amino acids (step "S"), and organic acids (step "W" in Figure 8) in the buds were extracted by the method described in Chapter IV and summarized in Figure 8. Radioactivity in each fraction along the line of the extraction as shown in Figure 8 was measured by withdrawing 1 ml aliquot, mixing with 10 ml Aquasol-2, and counting by liquid scintillation spectrometry. Radioactivity in needles and bud residue after alcohol extraction was determined following combustion of the tissue.

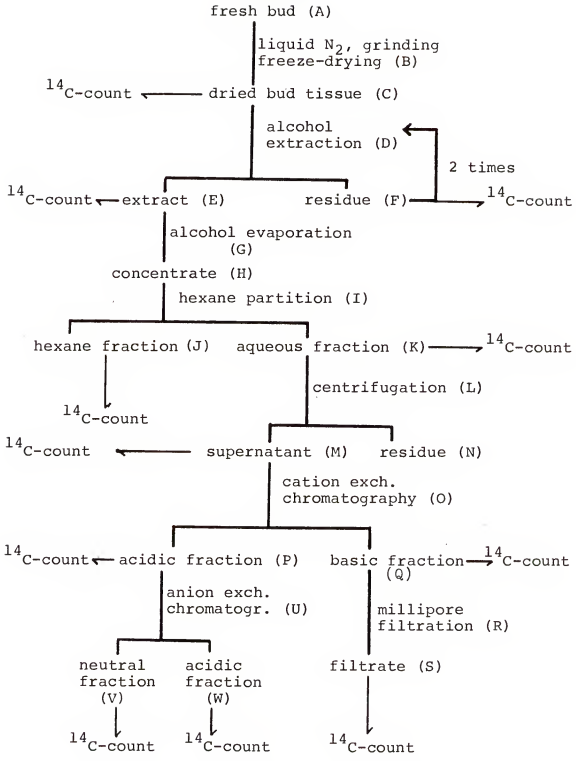


Figure 8. Extraction procedures for free sugars, organic acids, and free amino acids and determination of radioactivity in their fractions of Pinus elliottii bud tissue.

Results

Recoveries of known amounts of ^{14}C-sucrose and ^{14}C-arginine were 97.6% and 88.5%, respectively.

1975 Experiments

Distribution of ^{14}C (total radioactivity) in needles, stem, and bud tissue is shown in Figure 9. Radioactivity was detected in stems and terminal buds within 24 hours (Figure 9C and 9E). Radioactive photoassimilates in needles were transported to stems and terminal buds, resulting in a decrease in radioactivity in the needles (Figure 9A and 9B) and an increase in the terminal buds (Figures 9E and 9F) over the seven-day period. Basipetal movements of radioactivity toward the main trunk were not followed in this experiment, since acropetal translocation of ^{14}C from needles to terminal buds where floral primordia were initiated was of prime interest.

Radioactivity in terminal buds seven days after the exposure to $^{14}CO_2$ (Figure 9F) was much higher than in stem sections (Figure 9D). This suggested that radioactive materials were actively accumulated in the terminal buds, rather thanpassively moved along the concentration gradient. Buds of the poor-flowering group (PFG) showed higher radioactivity than those of the abundant-flowering group (AFG) both 24 hours and 7 days after the exposure (Figures (9E and 9F), indicating that photosynthate in the PFG was translocated to the terminal buds much faster than those in the AFG.

The ^{14}C-compounds in the terminal buds were fractionated into ethanol-soluble and ethanol-insoluble fractions, and the results are shown in Figure 10. Twenty-four hours after the exposure, about 85-90% of the total radioactivity of the buds was in the ethanol-soluble fraction in both flowering groups (Figures 10A and 10C). Soluble fraction of PFG 24 hours after exposure to $^{14}CO_2$ was more heavily labeled than that of the AFG (Figure 10A). Radioactivity in the soluble fraction after seven days decreased to 30-40% of total radioactivity in the bud and appeared to be the same in both flowering groups (Figure 10B).

The insoluble fraction showed an opposite trend to the soluble fraction in the distribution of radioactivity over the seven-day period. Radioactivity after 24 hours was similar in the two groups (Figure 10C), whereas radioactivity after seven days was higher in the PFG than in the AFG (Figure 10D). Thus, the terminal buds of the PFG, judged by a short-term (24 hours, Figure 10A) and long-term (7 days, Figure 10D) observations, received more radioactive photosynthate from the needles than the buds of the AFG. But a higher percentage of the photoassimilate was immobilized in the PFG than in the AFG over the seven-day period.

When the ethanol-soluble fraction was further separated into carbohydrate and amino acid fractions, radioactivity of both fractions in the buds of the PFG was higher than in those of the AFG both 24 hours and 7 days after the exposure (Figure 11).

Figure 9. Distribution of radioactivity (total radioactivity) in needles, stem, and buds of abundant-flowering (AFG) and poor-flowering group (PFG) of Pinus elliottii 1 day and 7 days after current-year needles were exposed to 50 μCi of $^{14}CO_2$ for 90 minutes. Each point is an average of 4 observations from 4 different trees.

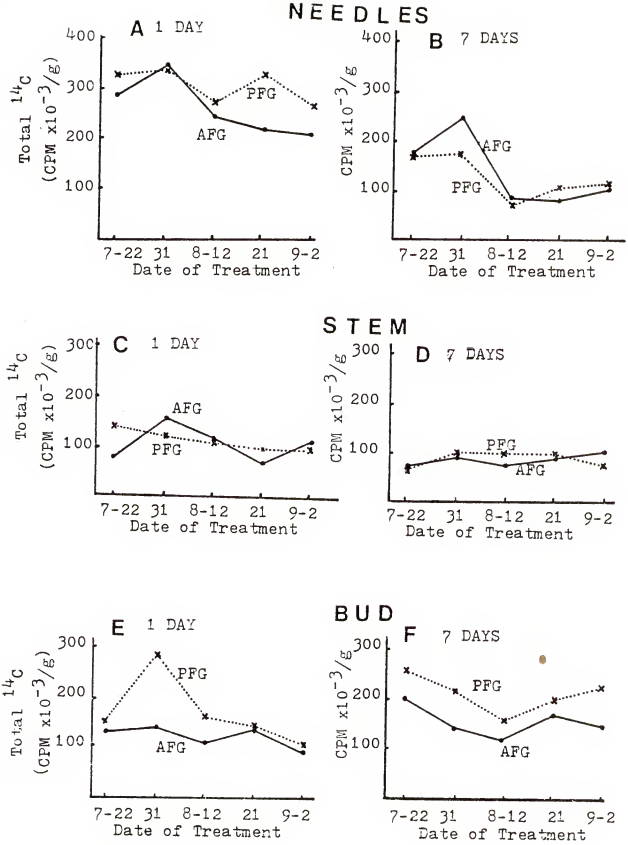
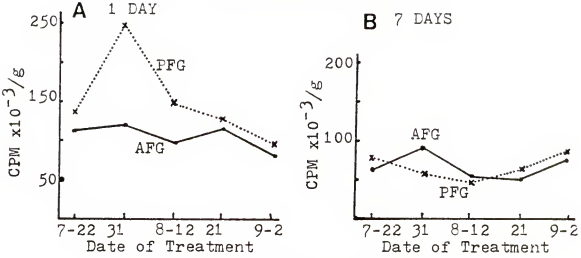

NEEDLES
A 1 DAY
B 7 DAYS
Total ^{14}C (CPM x10^{-3}/g)
CPM x10^{-3}/g
400
300
200
100
PFG
AFG
7-22 31 8-12 21 9-2
Date of Treatment
STEM
C 1 DAY
D 7 DAYS
BUD
E 1 DAY
F 7 DAYS

Figure 10. Distribution of radioactivity in ethanol-soluble and ethanol-insoluble fractions of bud tissue from abundant-flowering (AFG) and poor-flowering group (PFG) of Pinus elliottii 1 day and 7 days after current-year needles were exposed to 50 μCi of $^{14}CO_2$ for 90 minutes. Each point is an average of 4 observations from 4 different trees.

ETHANOL-SOLUBLE FRACTION



ETHANOL-INSOLUBLE FRACTION

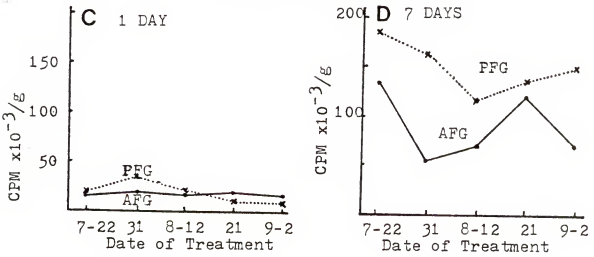
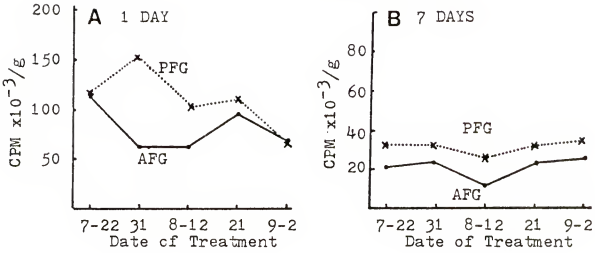
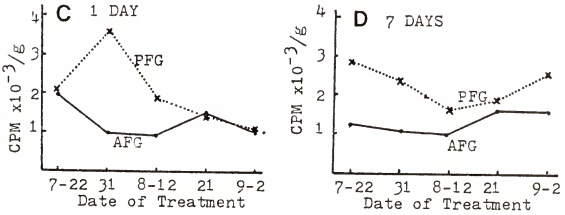


Figure 11. Distribution of radioactivity in free sugar and amino acid fractions of bud tissue from abundant-flowering (AFG) and poor-flowering group (PFG) of Pinus elliottii 1 day and 7 days after current-year needles were exposed to 50 μCi of $^{14}CO_2$ for 90 minutes. Each point is an average of four observations from four different trees.

SUGAR FRACTION



AMINO ACID FRACTION



1976 Experiments

Distribution of total radioactivity in the needles, buds, and various fractions of bud extract is shown in Figure 12. Radioactivity in the needles (Figure 12A) of the large buds of AFG during the first exposure was greater than in the needles of the three other types of buds, but no difference was observed during the second exposure. The bud tissue (Figure 12B) during the first exposure on July 28 showed no difference in radioactivity among the four types of buds, whereas radioactivity during the second exposure on August 26 was highest in the small buds of PFG, and second highest in the small buds of AFG. The large buds of the AFG had the lowest radioactivity among the four types of buds.

When radioactivity in the bud tissue was divided into ethanol-soluble and ethanol-insoluble fractions (Figures 12C and 12D), over 85% (an overall average of four types of buds) of total activity one day after exposure and 55% of activity seven days after exposure remained in the ethanol-soluble fraction (Table 8). Radioactivity of the ethanol-soluble fraction was higher in the small buds than in the large buds of either flowering group (Figure 12C).

The ethanol-soluble fraction was further fractionated into hexane-soluble, amino acid, sugar, and organic acid fractions (Figures 12E through 12H). Radioactivity in sugars over the seven-day period decreased, while activity in the hexane fraction, amino acids, and organic acids increased during the same period except for amino acids and

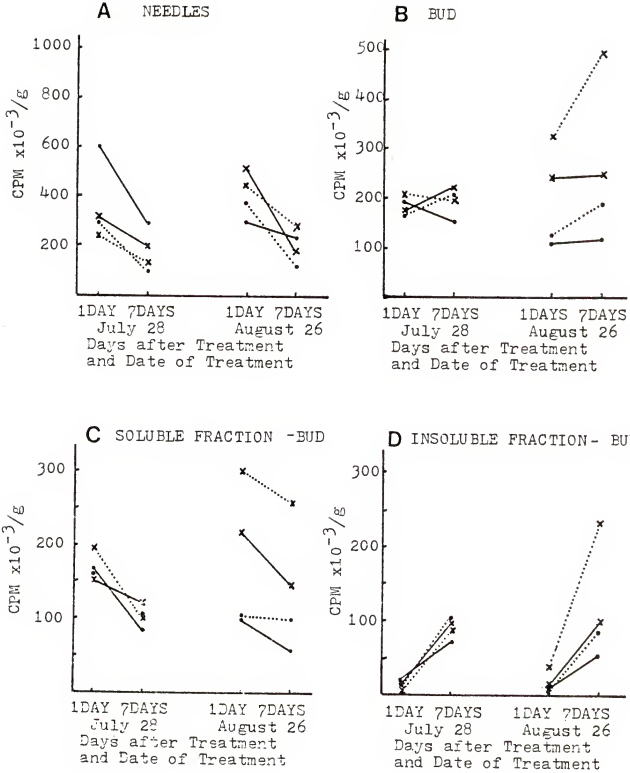
organic acids in the large buds of AFG in July (Figures 12F and 12H). Radioactivity in various fractions of the small buds were at all times higher than those of the large buds from either flowering group.

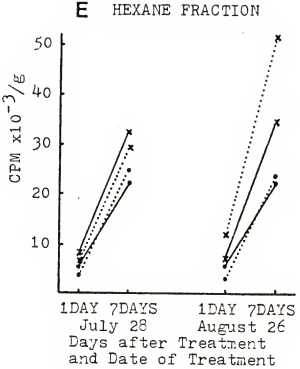
When radioactivity in the various fractions was expressed as percent of total radioactivity of bud, little difference was observed among the four different types of terminal buds at either treatment date (Table 8). However, hexane-soluble fraction showed somewhat higher percent in large buds of AFG in the August 26 treatment than in other bud types.

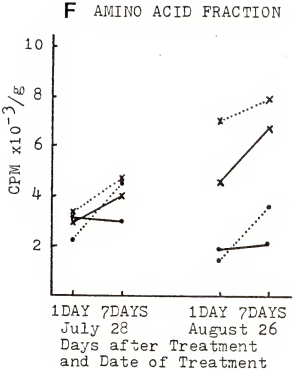
Figure 12. Distribution of radioactivity in needles, bud, and various fractions of bud extract from abundant-flowering and poor-flowering groups of Pinus elliottii 1 day and 7 days after current-year needles were exposed to 50 μCi of $^{14}CO_2$ for 90 minutes. Each point is an average of two observations from two different trees.

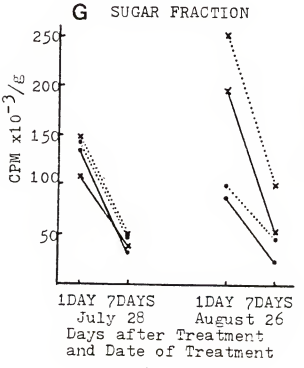
Line identities:

•——•: abundant-flowering group, large bud
×——×: abundant-flowering group, small bud
•·····•: poor-flowering group, large bud
×·····×: poor-flowering group, small bud


A NEEDLES
CPM x10^{-3}/g
1000
800
600
400
200
1DAY 7DAYS
July 28
1DAY 7DAYS
August 26
Days after Treatment
and Date of Treatment
B BUD
CPM x10^{-3}/g
500
400
300
200
100
1DAY 7DAYS
July 28
1DAYS 7DAYS
August 26
Days after Treatment
and Date of Treatment
C SOLUBLE FRACTION -BUD
CPM x10^{-3}/g
300
200
100
1DAY 7DAYS
July 28
1DAY 7DAYS
August 26
Days after Treatment
and Date of Treatment
D INSOLUBLE FRACTION- BUD
CPM x10^{-3}/g
300
200
100
1DAY 7DAYS
July 28
1DAY 7DAYS
August 26
Days after Treatment
and Date of Treatment


E HEXANE FRACTION
CPM x10⁻³/g
50
40
30
20
10
1DAY 7DAYS
July 28
1DAY 7DAYS
August 26
Days after Treatment
and Date of Treatment


F AMINO ACID FRACTION
CPM x10⁻³/g
10
8
6
4
2
1DAY 7DAYS
July 28
1DAY 7DAYS
August 26
Days after Treatment
and Date of Treatment


G SUGAR FRACTION
CPM x10⁻³/g
250
200
150
100
50
1DAY 7DAYS
July 28
1DAY 7DAYS
August 26
Days after Treatment
and Date of Treatment

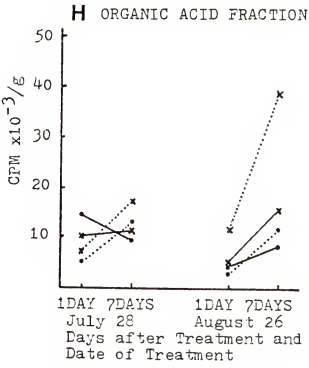

H ORGANIC ACID FRACTION
CPM x10⁻³/g
50
40
30
20
10
1DAY 7DAYS
July 28
1DAY 7DAYS
August 26
Days after Treatment and
Date of Treatment

Table 8. Distribution of radioactivity (percent of total radioactivity of bud) in ethanol-soluble, hexane-soluble, amino acid, sugar, and organic acid fractions of bud extract from abundant-flowering (AFG) and poor-flowering groups (PFG) of Pinus elliottii 1 day and 7 days after current-year needles were exposed to 50 μCi of $^{14}CO_2$ for 90 minutes. The ethanol-soluble fraction was fractionated into hexane-soluble, amino acid, sugar, and organic acid fractions.

fraction	flowering group	bud size	date of treatment: July 28, 1 day	July 28, 7 days	August 26, 1 day	August 26, 7 days
ethanol-soluble	AFG	large	89.5	53.3	90.9	50.0
	PFG	large	84.2	70.9	84.6	54.1
	AFG	small	88.2	53.3	91.7	59.2
	PFG	small	92.8	51.3	90.9	54.2
hexane-soluble	AFG	large	2.6	15.0	5.5	19.2
	PFG	large	2.4	12.2	2.4	12.9
	AFG	small	4.6	15.0	3.1	14.0
	PFG	small	3.3	14.9	3.6	10.8
amino acid	AFG	large	1.7	2.0	1.6	1.7
	PFG	large	1.4	2.2	1.1	1.9
	AFG	small	1.6	1.8	1.8	2.7
	PFG	small	1.6	2.5	2.1	1.6
sugar	AFG	large	68.4	20.0	75.4	18.3
	PFG	large	84.8	21.9	80.0	23.2
	AFG	small	62.9	15.9	77.6	22.0
	PFG	small	69.0	25.6	75.8	20.8
organic acid	AFG	large	7.9	6.0	3.6	6.7
	PFG	large	3.0	6.7	2.4	6.5
	AFG	small	5.7	5.0	2.0	6.4
	PFG	small	3.3	8.7	3.5	7.9

Discussion

In the 1975-experiments (Figures 9A and 9B) when the current-year needles were exposed to $^{14}CO_2$ in July (July 22 and July 31), less than 35% of the total radioactivity was transported out of the needles seven days after the exposure, indicating that the current-year needles utilized the majority of the photosynthate for their active elongation. When the needles were exposed to $^{14}CO_2$ in August or September, on the other hand, more than 65% of the total activity was moved out of the needles seven days after the exposure. This indicated that current-year needles had already elongated substantially by late August and were able to contribute a majority of their photosynthate to the other parts of the plant.

In the 1976-experiments, the greater radioactivity per g dry weight in the small buds of both flowering groups than in the large buds (Figure 12B) suggests that the small buds act as active sinks for photosynthate in late August and early September when male strobilus primordia are under active differentiation. The small buds of the PFG, which produce greater numbers of male strobili than the small buds of the AFG (as shown in Table 4), were also stronger sinks than the small buds of the AFG, suggesting a quantitative relationships between the number of developing male strobilus primordia and the amount of photosynthate required forthe developing primordia.

The low radioactivity per g dry weight in the large buds of the AFG suggests that the capacity of these buds to act as metabolic sinks might be reduced druing the period of initiation of female strobilus primordia. These buds do not initiate male strobilus primordia and, thus, do not require by late August as much photosynthate as the small buds.

In Pinus elliottii, whose pattern of shoot development features "free growth" (elongation of a shoot due to the simultaneous initiation and elongation of new stem units) as well as "fixed growth" (elongation of predetermined stem units) (Lanner, 1976), the last free growth, if more than one, in a growing season is followed by termination of elongation growth and reduction in metabolic activity of the terminal buds prior to the initiation of female strobili. The low radioactivity in the large buds of the AFG directly reflects the low metabolic activity of these buds. This point is further supported by the low amino acid contents in the large buds of the AFG as shown in the preceding chapter. Lang (1965) stated that the temporary cessation of any growth activity in the apical meristem was the first effect of photoinduction in many herbaceous plants and was followed by transition from vegetative growth to flower initiation.

The large buds of the AFG were on branches bearing first- and second-year strobili which require a large amount of food for their expansion and maturation. Actively elongating cones (second-year cones in the AFG) were shown to be

strong sinks for photosynthate. Dickmann and Kozlowski (1968, 1970) found second-year cones to be the strongest sink for ^{14}C-labeled photosynthate in Pinus resinosa. Thus, large buds of the AFG seemed to receive less amount of ^{14}C from needles than the small buds, and photosynthate from needles of the AFG was probably transferred to developing cones.

CHAPTER VI
CONCLUSIONS

Counts of female and male strobili produced in the abundant-flowering and poor-flowering groups of Pinus elliottii showed a strong genetic influence on flowering. Potential of trees to produce female cones appeared to be biologically fixed and controlled by unknown internal conditions.

Total needle weight per shoot appeared to be qualitatively as well as quantitatively associated with flowering, as judged by the tendency for transition from male to female flowering (a qualitative change) to be associated with increasing vigor of the shoot and also judged by the positive correlation of the number of female flowers (a quantitative measurement) with needle weight.

Results of the analysis of free sugars and free amino acids in the terminal buds during the period of floral bud initiation suggested that female flower initiation was associated with a low supply of nitrogen during the time when the supply of carbohydrates was not limiting. This might lead to "a nutritional stress" which might be responsible for the transition of the meristem to a reproductive phase.

The low arginine concentration in the female-producing buds suggested a minimal or negative role of arginine in the floral bud initiation of Pinus elliottii. It is likely that accumulation of arginine reported in the literature (in the cases where nitrogen fertilizer was used to stimulate female flowering) may simply reflect the excess nitrogen in the tissue after heavy fertilization.

A review of literature on the physiological conditions of flower initiation in conifers and on the flower induction by silvicultural treatments strongly suggested that flowering was associated with unbalanced nutrition between carbohydrates and nitrogen. An explanation for effectiveness of various silvicultural treatments in flower induction may be timely application of "stress" to trees, which might temporarily reduce the metabolic activity of the terminal buds. The low ^{14}C-translocation in the female-producing buds also suggested that metabolic turnover of ^{14}C-labelled photosynthate in the buds was reduced during the period of floral bud initiation. A future study of the rate of metabolism of ^{14}C-labelled photoshythate in the buds in the early growing season (period of rapid vegetative growth) and in the mid- to late summer (period of floral bud initiation) is suggested.

A mechanism may exist within a tree to measure the nutritional balance between carbohydrate and nitrogen availability, so that any external stimulus which disturbs this delicate balance, either increasing or decreasing the ratio

of carbohydrates to nitrogen, may drive the tree into "a nutritional stress." This nutritional or physiological stress may temporarily reduce the metabolic activity of the terminal buds and may affect a hormonal balance which in turn directs induction of floral bud primordia.

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